CRANFIELD UNIVERSITY

CRANFIELD HEALTH

PhD Thesis

Academic years 2006-2009

VASILIKI FRAGKOU

IMPROVEMENTS IN ELECTROCHEMICAL GLUCOSE BIOSENSORS

Supervisors:

Dr. Yi Ge

Prof. Sergey Piletsky

Prof. Anthony P.F. Turner

September 2010

This thesis is submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

ABSTRACT

Diabetes is one of the leading causes of death and disability in the world. Even though insulin was discovered in 1920, an intense research on diabetes has been conducted during the last five decades and this is because of the market size. The huge demand is creating the need for the development of new approaches. This project involved the research aimed at better understanding and improvements in performance of glucose biosensors.

In general, high surface area electrodes are desired as the high surface area provides more active sites for electrochemical reactions, and hence higher kinetic rate capability. Therefore, the determination of the active electrochemical surface area of the electrode is very important. A study has been conducted to determine the real electrochemical surface area of the Pelikan screen printed electrodes (SPEs) and a method has been optimised and established by Pelikan for the evaluation of their SPEs. Another very important issue that most of the current blood glucose monitoring tests are facing is the haematocrit effect, since the haematocrit differences observed in the blood samples can significantly affect glucose measurements. Therefore a study has been conducted in order to observe the absorption of the blood samples into the working electrode paste according to the haematocrit level.

The second part of the study included the characterisation of the novel conjugated polymer made of *N-(N, N'*-diethyldicarbamoyl ethyl amido ethyl) aniline (NDDEAEA), the optimization of the conditions for the electrochemical polymerization, their application in grafting and finally the development of NDDEAEA -based glucose biosensor. The new conducting polymer, acted as a matrix for the biosensor

fabrication in this study, possesses macroiniferter properties and is capable of initiation free radical-initiated addition polymerisation after formation of the polyaniline (PANI) material while preserving or even enhancing some of the PANI's electrochemical properties. This material can potentially be used in the construction of novel Pelikan electrodes with enhanced integration functionalities, e.g. grafting non-adhesive polymer coatings to assure that the poor performance in sensors as a result of impact of blood components can be mitigated.

The final study included the development and optimisation of the reaction conditions for grafting a hyperbranched polymer onto the surface of the multi-walled carbon nanotubes (MWCNT), using the A_3 and B_2 approach (described below). The aim of this work was achieving further increase in the sensitivity of Pelikan sensors.

ACKNOELEDGEMENTS

First and foremost I offer my sincerest gratitude to my supervisor, Prof A.P.F Turner for giving me the chance to fulfil my dream. I would also like to thank my two other supervisors, Dr. Yi Ge and Prof S. Piletsky for all their help and support.

I gratefully acknowledge the financial support and technical expertise provided by Dr. D. Freeman, Dr. N Bartetzko and the entire group at Pelikan in Germany.

I would also like to thank Paula and Petya for shown great friendship and put up with all my moans and groans.

Finally, I would like to thank my family, my parents, brothers and sister for their endless support, love and caring.

I would like to dedicate this thesis to my baby boy, my inspiration and to my loving husband Nik for his love, endless patience, understanding and absolute confidence in me.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

A: real electrochemical surface area of the electrode (cm^2)

AFM: atomic force microscopy

A_x: analytical signal of the unknown

A_{x+s}: analytical signal of unknown plus standard

C: the concentration of the mediator

CA: contact angle

CC: chronocoulometry

C_o: molar concentration of oxidised species

C_r: molar concentration of reduced species

C_s: standard concentration

CV: cyclic voltammogram

C_x: standard concentration of the unknown

D: diffusion coefficient

d: diffusion limited variable (Id diffusion current)

D_o: diffusion coefficient of oxidised species

E: electrode potential

E_p :the potential of the peak

F: Faraday's constant

Gox: glucose oxidase

HBP: hyperbranched polymers

I: current density

I_p :peak current

k: kinetic constant

MWCNT: multiwall carbon nanotubes

n: number of electrons exchanged within electrode reaction

NDDEAEA: *N-(N, N'*-diethyldicarbamoyl ethyl amido ethyl) aniline

PANI: polyaniline

PBS: phosphate buffer saline

PQQ: pyrroloquinolone quinone

Q: charge

R: universal gas constant

RESA: real electrochemical surface area

SEM: scanning electron microscopy

SPE: screen printed electrodes

T: temperature

t: time

TMPD: *N,N,N′,N′*-Tetramethyl-p-phenylenediamine

v: potential sweep rate

$\mathbf{V_s}$: standard volume

$\mathbf{V_x}$: volume of the unknown

WE: working electrode

RATIONALE FOR RESEARCH

Diabetes is one of the leading causes of death and disability in the world. 171 million people in the world are suffering from this disease and several studies have demonstrated the likely increase of this figure to 366 million by 2030. The quick diagnosis and early prevention are critical for the control of the disease. Forty years have passed since Clark and Lyons proposed the concept of glucose enzyme electrodes. Since then, excellent economic prospects and fascinating potential for basic research have led to many sensor designs and detection principles for the biosensing of glucose.

Because of this intense competition, the companies have resorted to a strategy where the existing technology is rapidly evolved and “re-packaged” to meet commercial objectives, such as small size and fast response and consequently the technology has moved far away from the theoretical understanding of the functionality of these devices, generating a serious gap in the knowledge required for further optimisation.

As this field enters the fifth decade of intense research, the market size and the huge demand are creating the need for the development of new approaches. This project was aimed at better understanding and improvements in performance of glucose biosensors, in particular these produced by Pelikan – sponsor of this work.

The first part of the study includes the study of different aspects of the Pelikan screen printed electrodes. More precisely the determination of their real surface area

and an investigation of the impact of major haematocrit effect on sensor response which most of the present commercial electrochemical glucose biosensors seem to present was carried out.

The second part of the study includes the characterisation of the NDDEAEA, the optimization of the conditions for the electrochemical polymerization, their application in grafting and finally the development of a glucose biosensor. The new conducting polymer N-(N, N'-diethyldicarbamoyl ethyl amido ethyl) aniline (NDDEAEA), which was characterized and acted as a matrix for the biosensor fabrication in this study, can act as a macroiniferter and is capable of electrochemical polymerisation to form PANI-based materials in various formats capable of utilization in other reactions, such as participation in free radical-initiated addition polymerisation after formation of the PANI material while preserving or even enhancing some of the PANI's properties.

The final study includes the development and optimisation of the reaction conditions to grafting a hyperbranched polymer onto the surface of the multi-walled carbon nanotubes (MWCNT), using the $A_3 + B_2$ approach.

1st CHAPTER

INTRODUCTION

1.1 BRIEF BACKGROUND

Diabetes is a metabolic disorder which occurs either because of a lack of insulin or because of the presence of factors that opposes the action of insulin. Diabetes is a chronic condition that without treatment can have very serious consequences. The health implications of diabetes are astounding, since this disease is associated with morbidity and mortality. Over time, diabetes can lead to blindness, kidney failure, and nerve damage. Diabetes is also an important factor in accelerating the hardening and narrowing of the arteries (atherosclerosis), leading to strokes, coronary heart disease, and other blood vessel diseases. (***1***) , (***2***) The care and management of diabetes involve heavy costs. It has been characterised as one of the largest therapeutic segments of global pharmaceutical sales. In 2005 the generated sales were over $15 billion and in 2009 it was valued at $25 billion. It should be noted that people with diabetes incur two to five times higher medical costs than people without diabetes. These medical costs include medical visits, admission to a hospital and the purchase of supplies and medications.

There is currently no actual cure for diabetes and the control of elevated blood glucose levels, without causing abnormally low levels, is the major goal in treating diabetes. This is now easily achieved with a blood glucose meter (glucose biosensor) and the invention of this technology was one of the most important steps in managing diabetes, since it also reduces the risk of long-term complications. In comparison with conventional methods of glucose testing, glucose biosensors

provide a rapid and easy method of testing blood glucose levels. This is one of the major reasons that the glucose biosensors occupy 85% of the current world market for biosensors and worth approximatelly $6.9 billion. (***3***) , (***4***) This figure will reach $11.5 billion by 2012 according to the Global Industry Analysts. (***5***) Currently, there are more than 56 commercialised devices for detecting blood glucose levels, manufactured by 18 different companies but four multinational companies dominate the biosensor industry: Roche Diagnostics, LifeScan, Abbott and Bayer.

Nowadays the glucose biosensors are more accurate and easy to use. Netherless are still less precise and accurate than the methods used in the laboratories. Therefore further improvements should be achieved.

1.2 DIABETES MELLITUS

Diabetes Mellitus (Diabetes) (***2***) is characterised by the presence of excess of glucose in the blood and tissues of the body. It is a metabolic disorder in which the pancreas underproduces or does not produce insulin. Insulin is a hormone produced by the pancreas that is required by cells of the body in order to use glucose, the major source of energy for the human body. There are three main types of diabetes: Type I diabetes, Type II diabetes and Gestational Diabetes. (***6***)

1.2.1 Type I diabetes

Type I diabetes was previously known as insulin-dependant diabetes and affects approximately 10% of all people with diabetes. It is rare in the first nine months of life and has peak incidences at twelve and between twenty to thirty five years old. (***7***) It is due to the destruction of beta-cells in the pancreatic islets of Langerhans with resulting loss of insulin production and finally in hyperglycaemia. The process of islets destruction probably begins very early in life and is known to start several years before the clinical onset of diabetes. Clinically a person with this disorder presents a number of symptoms, including: polydipsia (increased thirst), which is an attempt to compensate for fluid loss; polyuria (frequent passage of urine), which is due to the presence of excess glucose in the urine; muscle cramps, which are due to electrolyte disturbances because of the fluid loss; weight loss due to the lack of insulin and consequently to the break-down of proteins and fat despite increased appetite, blurred vision due to the excess accumulation of glucose in the eye lenses, nausea, vomiting and abdominal pain. (***8***)

1.2.2 Type II diabetes

Type II diabetes previously known as non-insulin dependent diabetes, affects approximately 90% of all people with diabetes and is usually found in middle aged or older patients. Type II diabetes is due to a combination of disorders with differing progression and outlook. This combination of disorders include resistance to the action of insulin in peripherical tissues such as muscle and fat cells, the failure of the insulin secreting cells of the pancreas to produce sufficient insulin and the failure of insulin to inhibit the production of glucose in the liver. Symptoms of people with type

II diabetes include: polydipsia, polyuria, increased appetite, fatigue, erectile dysfunction and frequent or slow-healing infections. (5)

1.2.3 Gestational Diabetes

Gestational diabetes occurs during pregnancy. The placenta, during pregnancy transfers nutrients to the fetus and also produces a number of hormones (e.g.cortisol and progesterone) that interfere with the body's usual response to insulin (insulin resistance). When pregnant women present high blood glucose levels because their pancreas cannot produce extra quantities of insulin in order to compensate for insulin resistance, then these women are consider to have gestational diabetes. Gestational diabetes affects two to four percent of pregnancies, depending on the population studied. (***9***) Gestational diabetes can be characterised as a treatable condition, since women can effectively decrease several risks attributed to the specific disease by controlling their blood glucose levels.

1.3 INCIDENCE - A MAJOR WORLD PROBLEM

Diabetes is a major world health problem. In 2000, it was estimated by the world health organisation, that the number of diabetics worldwide was 171 million and approximately 4 million deaths each year were caused by diabetes (9% of deaths worldwide). Several studies on the prevalence of diabetes have shown that the above figure of 171 million is projected to rise to 366 million by 2030. (***10***) These studies usually include data on diabetes prevalence according to age, sex and region. The prevalence of diabetes among people aged 20-79 years will be 6.4% affecting 285 million adults in 2010 and by 2030 this figure will increase to 7.7%,

affecting 439 million people. (***11***) The prevalence of diabetes is higher in men than women despite the fact that at the moment there is, worldwide, a significantly higher amount of women suffering from diabetes. (***12***) The same study also demonstrated that the most important demographic change to diabetes prevalence worldwide appears to be the increase in the proportion of people below the age of sixty-five years.

Diabetes was once considered to be a disease afflicting wealthy countries. (***13***) Nowadays, it is affecting low income and developing countries. In the future the largest proportional and absolute increase will occur in developing countries, where the prevalence will rise from 4.2% to 5.6%. (***14***) A recent study showed that between 2010 and 2030 there will be a 69% increase of diabetics in the developing countries and 20% increase of diabetics in developed countries. (***11***)

Regionally by 2025, the adult diabetic population is expected to double in India to about 73 million and in China to 46 million. It is worthy to mention that the 36% of the anticipated absolute global increase of diabetics will occur in India and China alone. (***11***) At the same time, diabetes prevalence is expected to increase to 2.8% of the adult population in Africa and 7.2% in South and Central America. The European Region, with 48 million, and Western Pacific Region, with 43 million, currently have the highest number of people with diabetes. However the prevalence rate of the Western Pacific Region at 3.1% is significantly lower than 7.9% in the North American Region and 7.8% in the European Region (Fig.1). (***15***), (***16***)

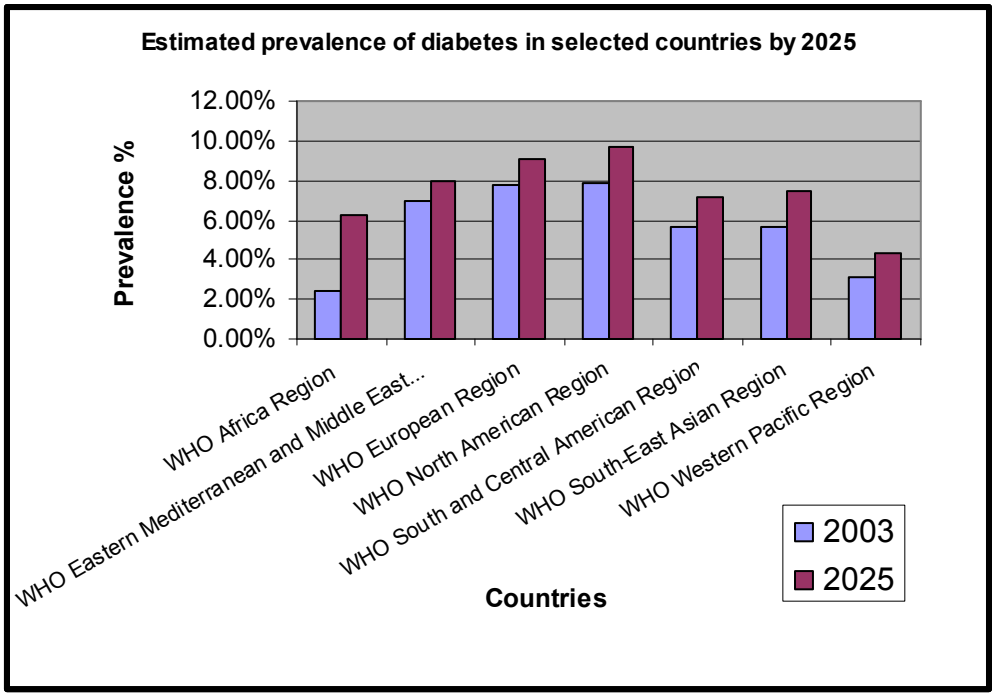


Figure 1: Estimated prevalence of diabetes in selected countries by 2025 **(***14***)**

It is worthy of note that by 2010, cases of type 2 diabetes are projected to rise 111%, from 62 million to 132 million, and in mainland China alone, the increase over the next 10 years is predicted to be 35-36 million. In general, worlwide, type 2 diabetes clearly predominates, but several reports clarify that the 4.3 million cases of type I diabetes are expected to increase to 5.3 million cases by 2010. (***15***)

1.4 TREATMENTS

Diabetes is a chronic condition that without treatment can have very serious consequences. The overall health implications of diabetes are astounding. The disease is associated with morbidity and mortality and studies have shown that

persons diagnosed with diabetes before the age of forty tend to present a life decrease, which for men amounts to 12 years and for women to 19 years.

The complications of diabetes could be mitigated by controlling the blood glucose levels. In general the type I diabetes can be treated with insulin, exercise and diet, while type II diabetes is initially treated with weight reduction, diet and exercise. However when all the above fail to control the high glucose levels, oral medications (e.g. metformin, sulphonyl ureas, thialzoldinediones, etc.) or even insulin are used. (***17***)

It is worthy to note that it is necessary not only to treat the diabetes but to monitor the effects of treatment on blood glucose levels to avoid overtreatment or undertreatment of the diabetes. (***18***)

1.5 HOME URINE/BLOOD GLUCOSEMONITORING

Urine and blood glucose self monitoring are the two primary methods used by the person with diabetes to monitor their diabetes control.

1.5.1 Urine glucose monitoring

Urine glucose monitoring (***19***), (***20***) is a viable, cost-effective way of monitoring diabetes control, but has a number of limitations and is not a substitute for blood glucose monitoring. There are two types of urine glucose tests, which are based on a chemical reaction. By placing a specific strip or tablet in a urine specimen a chemical reaction occurs resulting to a colour change according to the glucose level presented in the sample. The specific colour is then matched against to a colour chart provided by the manufacturer and the result is obtained. In more details the first type, the copper reduction test, uses cupric sulfate. In the presence of glucose, cupric sulfate, which is blue, changes to cuprous oxide, which is green to orange. The second type of urine glucose test, called the glucose oxidase test, uses the chemical toluidine and the enzyme glucose oxidase. Glucose oxidase converts the glucose in urine to gluconic acid and hydrogen peroxide. The interaction of the hydrogen peroxide with the toluidine causes a change in colour. The main limitations of this kind of test is firstly the fact that the cupric sulfate and the glucose oxidase can react with substances other than glucose in the urine and lead to false positive results, like aspirin, penicillin, vitamin C, and cephalosporin-type antibiotics and secondly it cannot distinguish between low and normal blood glucose levels, or high and very high blood glucose levels. However, urine glucose monitoring is particularly helpful in

persons at low risk of hypoglycaemia and whose blood glucose levels are not too high and generally stable. (***21***)

1.5.2 Blood glucose monitoring

Blood glucose monitornig (***22***), (***23***) can be achieved in several ways. The pricking of the finger with a small lancet in order to produce a droplet of blood, which is placed onto the strip and into a meter that displays the blood glucose level, is the most popular way of testing. In the commerce can be found several meters, presenting various features, sizes and costs, as well as alternative testing sites such as the upper arm, forearm, base of the thumb and thigh. It is worthy to mention though that testing at alternative sites, may give slightly different results than these obtained by the conventional fingertip pricking. (***24***)

1.6 GLUCOSE METERS

Before focusing on the glucose biosensors it is importamt to have a quick look in the past, in order to get a broader understanding of their importance.

1.6.1 Colorimetric Strips

Dextrostix was a paper strip developed and introduced to the market in 1965 by Ames, a division of Miles laboratories. By adding one drop of blood on the strip, a blue colour was being developed which was interpreted by comparing it to a colour chart, which was indicating an approximation of the blood glucose level.

People who were following this procedure on a regular basis frequently got to read Dextrostix strips very well. But for most people because of the limited usage, the interpretation wasn't always successful. (***25***), (***26***), (***27***), (***28***).

1.6.2 Ames Reflectance Meter

The solution came with the development of the Ames Reflectance Meter in 1971 by Anton H. Clemens. The device was able to read and interpret the Dextrostix strips. In more details the Ames Reflectance Meter was a light meter that read reflected light. This reflected light was sent to a photoelectric cell and a read out was given. Although the instrument was more able to read the minor changes in the reflection of the darkness and lightness of the shade of blue, adding a certain degree of interpretation accuracy, was very expensive, heavy, requiring also connection to an electrical outlet for power and most important a prescription. It is worthy to note though that this instrument was the pioneer which eventually led to a variety of other products. (***29***)

1.7 GLUCOSE BIOSENSORS

1.7.1 Clark Oxygen Electrode

Prof. Lelend Clark Jnr was immortalised by his invention of the Clark Oxygen Electrode in 1956.(Fig.2)

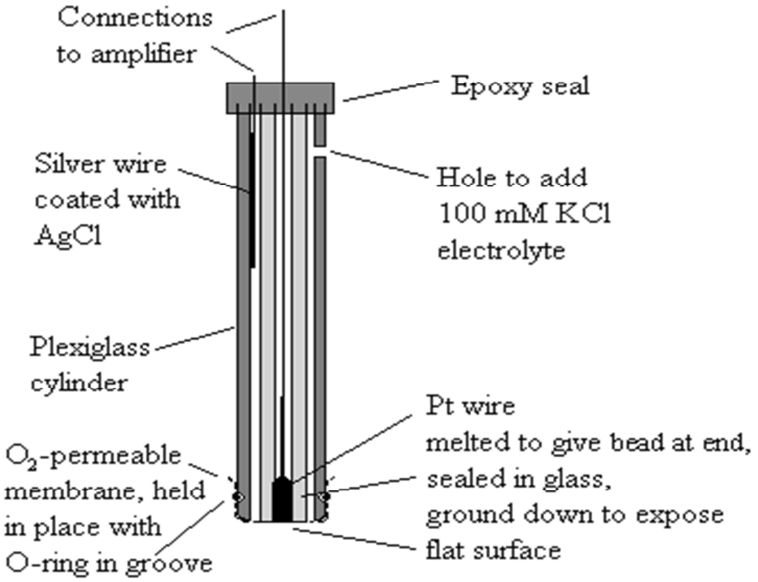


Figure 2: Clark Oxygen Electrode **(***30***)**

A few years later in 1962 at a New York Academy of Sciences symposium Clark described how "to make electrochemical sensors more intelligent, "by adding" enzyme transducers as membrane enclosed sandwiches". By performing an experiment, he managed to entrap glucose oxidase at a Clark oxygen electrode by using a dialysis membrane and demonstrated that the decreased measured oxygen was proportional to the glucose concentration. The sensor invented by Clark

comprised the basis of numerous variations on the basic design and many other oxidase enzymes were immobilised by various workers in subsequent publications. (***30***)

In nature, glucose oxidase as well as other oxidase enzymes act by oxidising their substrates, accepting electrons in the process and thereby changing to an inactivated reduced state. The purified enzymes are normally returned to their active oxidised state by transferring these electrons to molecular oxygen, resulting in the production of hydrogen peroxide. (Fig.3)

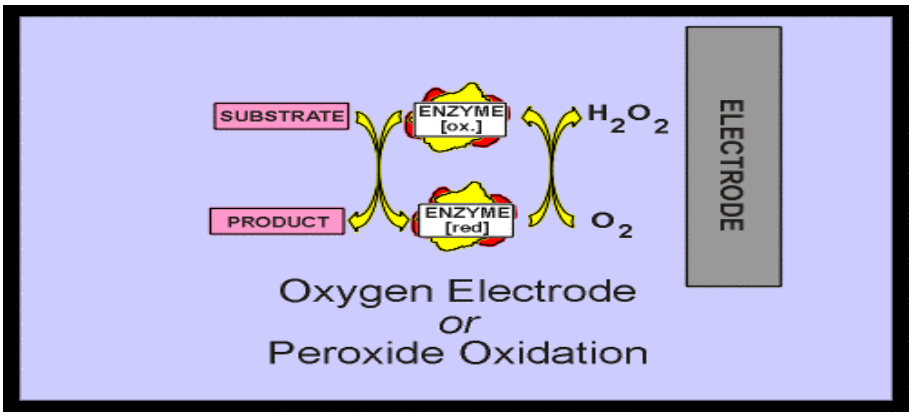


Figure 3: First generation glucose biosensor schematic **(*4*)**

It is worthy to note at this point that the glucose concentration can be also measured amperometrically at a potential of +0.7V versus a silver-silver chloride electrode when a platinum working electrode is used, normally under strongly alkaline conditions.

1.7.2 Yellow Springs Instrument

In 1975 the construction of biosensors based on the Clark's approach became a commercial reality with the successful relaunch of the Yellow Springs Instrument. (***3***) The amperometric detection of hydrogen peroxide consisted the fundamental basis of these instruments.

While they remained in the market eversince and they are met in hospitals and laboratories in all over the world, over the years the biosensor constuction hasn't changed significantly. The basic format is based on the immobilisation of glucose oxidase between two membrane layers. The outer polycarbonate membrane retains the enzyme, allowing glucose to pass, but prevents many larger molecules from entering, reducing the interference. The glucose enters the enzyme layer, where it is oxidised, and hydrogen peroxide is produced. This passes through the cellulose acetate membrane to a platinum electrode where it is measured amperometrically. The second membrane acts as a further size exclusion barrier preventing many other potentially interfering (electroactive) compounds from reaching the electrode surface. What has been concluded by the construction of these devices is that they are robust but not straightforward to miniaturise, expensive and finally the high detection voltage required makes the system prone to interference in the absence of the membrane structures utilised. (***31***)

1.7.3 Mediated Biosensors

The problems related to the sensitivity of O_2 concentration and the interference signals produced by ascorbate, urate and paracetamol were overcomed with the use of some compounds known in our days as mediators. (***32***)

Around 1980 a group of scientists based at Cranfield and Oxford Universities, working on fuel cells realised that the electron transfer compounds used for their projects could have a promising use in the biosensor application. (***33***), (***34***)

The mediators can shuttle electrons between the redox center of the enzyme and the electrode. The most important examples of these mediators, are mediators based on the ferrocene and its derivatives, since they have a wide range of redox potentials.

One of the most important breakthroughs was the incorporation of the mediators in the glucose biosensors, since they superceded the reflectance devices for home blood glucose testing.

It has to be noted though that one of the major drawback of the mediators is the fact that they are relatively soluble, resulting in short operational lifetimes and irreproducible results. In the case of the blood glucose sensing though this is acceptable since for the one shot use they have been proved robust and accurate. (***35***)

1.7.4 General principles

But what exactly is the biosensor and which are the key components for its functionality? “A biosensor is an analytical tool or system consisting of an immobilised biological material which converts information about the properties of the analyte into a quantifiable signal via a suitable transducer.” (***36***) In principle many transducers can be used in a biosensor for the measurement of glucose but, electrochemical methods have dominated the market, mainly because of the sensitivity, reproducibility, the inexpensive manufacture that they can offer and the easy maintance. (***5***) The most often electrochemical method employed is the amperometry, which relies on the application of a potential between two electrodes and the measurement of the resultant current flow. This is easily achieved in a three electrode cell, composed by a reference electrode, typically a Ag/AgCl electrode, a working electrode, which is usually made of a noble metal or carbon and a counter or auxillary electrode.

1.7.4a Enzyme

Another key component in the manufacture of a glucose biosensor is the enzyme. The most commonly used enzymes in the design of glucose biosensors contain redox groups that change redox state during the biochemical reaction. (***37***) Enzymes of this type are glucose oxidase and glucose dehydrogenase, which have a wide application in the commercial devices. The enzymes are proteins, consisted of one or more polypeptide chains with high molecular weights. The most significant property is the fact that they are highly specific in relation to the substrate molecules. The chemical bonding, the physical adsorption and the entrapment in a polymeric

matrix are the three main immobilisation methods used for the attachment of the enzyme to the transducer.

1.7.4b Mediators

As has been mentioned earlier, the performance of the glucose biosensors is highly linked with the mediators employed. (***38***) Some of the most significant examples of mediatord found in the commercial blood glucose sensors are the ferrocene and its derivatives, ferricyanide, osmium and TMPD. (Table 1)

Table 1: Examples of mediators

Device Name	**Mediator**
Precision	Ferrocene
Elite	Ferricyanide
Sure Step	Ferricyanide
AccuCheck	Ferricyanide
FreeStyle	Osmium
Pelikan Sun	TMPD

1.7.4c Polymers

Blood is a physiological material containing solutes and various species that might be electrochemically active and interfere with the glucose measurements. These substances are being binded to the electrode surface, causing the fouling or the loss of the sensor response. These problems could be solved by the application of a permselective coating to the sensor. These permselective coatings are known as polymers. Polymers are also used in order to entrap a mediator or a biological material close to the surface of an electrode. (***39***)

There are many different types of polymers but several studies have demonstrated that the conducting polymers are suitable for the immobilisation of enzymes at electrode surfaces offering an additional advantage of electron transport between enzyme catalytic centre and electrode. (***40***), (***41***), (***42***) By applying the correct conditions, several monomers can be electrochemically polymerised on the electrode surface and form conductive films. The biological material (biomolecule) can be entrapped in the conducting film if it is present in the solution during the electrochemical polymerisation or alternatively it can be adsorbed or chemically grafted after the initial electropolymerisation of the conductive polymer. (***43***), (***44***), (***41***), (***42***)

One of the most significant and widely used, conductive polymer is the polyaniline and its derivatives, because of the exceptional electrical, electrochemical and optical properties. (***45***) These are conjugated polymers which have the capability to conduct electrical charge and have been used both as an immobilisation media as well as a

physiological transducer, transforming the chemical signal into an electrical. (***46***) Their electrical conductivity is based on the electronic structure of their polymeric blackbone, which varies according to the applied potential and can be explained by the polaron formation. (***47***), (***48***) Upon oxidation, double bonds along the chain are broken leaving a radical and a positive charge unit (polaron) on the polymer chain. After further oxidation more polarons are formed along the chain and when the polaron concentration gets high enough the radical cations spread out through adjacent π structures across approximately eight bond lengths. The combination of two radicals, one from each polaron forms a new π bond. This results in the formation of a bipolaron which is more stable than two polarons than same distance apart. The bipolarons are free to move along the polymer chain and this action gives rise to electronic conductivity. It is also worthy to note that it is at this point in the oxidation process that the conductivity undergoes a marked increase. (***48***)

1.8 CURRENT COMMERCIAL HOME BLOOD GLUCOSE MONITORING

Biosensors can be developed for many different applications. (***49***) They have been applied to a wide variety of analytical problems included in: medicine and healthcare, environmental protection and biotechnology. (***50***) The largest market opportunity is represented by the biomedical area and this is one of the major reasons that the biosensor research and development has been largely concentrated in the specific area. A single analyte dominates this market and this is glucose. Blood glucose sensors account for 85% of the current world market for biosensors and are the most widely studied and commercialised of all biosensors. (***30***)

Commercial blood glucose meters are produced by many companies worldwide. (Table 2) Although the biosensor industry is dominated by several multinational companies, the major players are Roche Diagnostics, LifeScan, Abbott and Bayer. (***30***)

Table 2: Market leading glucose biosensor companies in 2003 (***30***)

Company Name	**Annual Sales** (Millions)
Roche Diagnostics	$ 1494
LifeScan	$1288
Abbott	$977
Bayer Diagnostics	$460
Becton Dickinson	$23
Hypoguard	$19

1.8.1 Roche Diagnostics

Roche Diagnostics is one of the major healthcare companies, worldwide and now offers a range of biosensor products to researchers, physicians, patients, hospitals and laboratories. In May of 1997, Roche re-entered the biosensor market (after an early unsuccessful foray into the area in 1977) by purchasing all shares of the diagnostics and pharmaceuticals businesses of the Boehringer Mannheim Group, which had a successful biosensor programme. The purchase price was around 11 billion US dollars. Roche Diagnostics reports that it now conducts research mainly into biosensors that permit near-painless, continuous measurement of blood glucose

level. It acquired from Boehringer Mannheim the very successful Accu-Chek family of products for blood-glucose monitoring. The blood glucose systems for patient self-testing comprise blood glucose monitors (Table 3), test strips and lancing devices as well as diabetes data management for professional use. It is worthy to note that Roche has one of the few meters with an integrated lancing device. The Accu-Check Compact Plus has an integrated 17 test drum, which eliminates the need for strip handling. (***30***), (***51***) However, the *Compact* still uses the last generation colorimetric strip technology while the *Aviva, Active, Go* and *Advantage* use the more modern electrochemical biosensor approach.

Table 3: Blood glucose meters by Roche Diagnostics

Device	**Sample Volume**	**Time**	**Specifications**
Accu-Chek Aviva System	0.6 uL	5 sec	1. Review of past results right on the meter 2. Audible test reminders
Accu-Chek Active System	1 uL	5 sec	1. Large memory capacity with averaging the results 2. Underdosing detection

Accu-Chek Compact Plus System	1.5 uL	5 sec	1. Integrated test strips 2. Integrated Safety
Accu-Chek Go System	1.5 uL	5 sec	1. Acoustic test reminder 2. Hypoglycemia threshold warning
Accu-Chek Compact System	1.5 uL	8 sec	1. Large memory capacity with averaging 2. Maximum safety
Accu-Chek Advantage / Accu-Chek Sensor System	4 uL	40 sec	1. Review of past results right on the meter 2. Acoustic test reminder 3. Visual hypoglycemia warning
Accu-Chek Complete System	4 uL	40 sec	1. Combines all information pertaining to blood sugar, insulin doses, carbohydrates, ketones, HbA1c, exercise, and event markers 2. Large memory capacity with averaging

1.8.2 LifeScan

LifeScan is part of the Johnson & Johnson family. The company became part of the biosensor market in 2001 by the acquisition of the diabetes care business of Inverness Medical. (***30***), (***52***) LifeScan presents an array of products for blood glucose testing on the market under the umbrella of One Touch products, like blood glucose meters (Table 4), test strips, lancets and lancing devices and diabetes management software. (***53***) The low volume, high performance devices are based on electrochemistry, while the *Basic* and *SureStep* still use colour reflectance technology.

Table 4: Blood glucose meters by LifeScan

Device	**Sample Volume**	**Time**	**Specifications**
OneTouch Ultra 2	1 uL	5 sec	1. Multiple test sites
OneTouch UltraSmart	1 uL	5 sec	1. Multiple test sites 2. Test results are entered automatically into 9 charts and graphs
OneTouch UltraMini	1 uL	5 sec	1. Small size
OneTouch Ultra	1 uL	5 sec	1. Multiple test sites
OneTouch Basic	10 uL	45 sec	1. Test memory with date and time
OneTouch SureStep	10uL	15 sec	1. Test memory with date and time 2. Automatic 14-day, 30-day test averages

1.8.3 Abbott

Abbott is a global, broad-based health care company supplying a range of products for the diagnosis and treatment of diseases. In 1996, it acquired MediSense for $867 million, which was the first company to commercialise home blood glucose biosensor technology. Following the purchase of two further biosensor companies, Therasense for $1.2 billion and i-STAT for $392 million in early 2004, Abbott has cemented itself in the top three largest biosensor companies worldwide. (***30***), (***54***) The company offers a range instruments for blood glucose testing, including blood glucose meters (Table 5), strips and lancets. (***55***)

Table 5: Blood glucose meters by Abbott

Device	**Sample Volume**	**Time**	**Specifications**
FreeStyle Flash	0.3 uL	5 sec	1. Alternative site testing 2. Presents 4 programmable alarms remind patients when to test
FreeStyle Mini	0.3 uL	7 sec	1. Alternative site testing 2. Presents 4 programmable alarms remind patients when

			to test 3. Large memory capacity 4. Automatic 14-day test averages
Precision Xtra	0.6 uL	5 sec	1. Auto Calibration 2. Simple blood calibration
Optium Xceed with Optium Xceed Plus test strips	0.6 uL	5 sec	1. Large memory capacity 2. Automatic 7-day, 14-day, 30-day test averages 3. Blood ketone testing 4. Alternative site testing
Optium Xceed w/o Optium Xceed Plus test strips	1.5 uL	10 sec	1. Large memory capacity 2. Automatic 7-day, 14-day, 30-day test averages 3. Blood ketone testing 4. Alternative site testing
MediSense Optium	25 uL	20 sec	1. Alternative site testing 2. Blood ketone testing

MediSense Soft-Sense		20 sec	1. Alternative site testing 2. Large memory capacity

1.8.4 Bayer HealthCare

Bayer HealthCare is a health care company of international acknowledgment. The Diabetes Care Division is one of the world's market leaders in the field of blood glucose monitoring systems and it markets, under the umbrella brand of Ascenia, blood glucose meters (Table 6), test strips and other accessories and services for blood glucose monitoring. Bayer acquired its biosensor technology via a Japanese alliance with Kyoto Daiichi. It is worthy of note that at the begining of 2003 the Diagnostics Division of Bayer HealthCare LLC and Matsushita Electric Industrial Co. Ltd. expanded their relationship with the signing of a joint venture agreement and establish "Viterion TeleHealthcare LLC", a Bayer-Panasonic company, to market products and services for the rapidly growing telehealth market. (***4***), (***56***)

Table 6: Blood glucose meters by Bayer

Device	**Sample Volume**	**Time**	**Specifications**
Ascenia Contour	0.6 uL	15 sec	1. Alternative site testing 2. Large memory capacity 3. No coding required
Ascenia Elite	2 uL	30 sec	1. 20 tests saving information with dates and times 2. “Sip-in Sampling” 3. Alternative site testing
Ascenia Elite XL	2 uL	30 sec	1. 120 tests saving information with dates and times 2. “Sip-in Sampling” 3. Alternative site testing
Ascenia Breeze	2.5-3.5 uL	30 sec	1. Large memory capacity 2. “Autodisc” 3. Alternative site testing 4. No coding required

1.8.5 Integrated devices

Pelikan Technologies in Palo Alto, California has developed the first, fully integrated, blood sampling and glucose measurement device. It is called Pelikan Sun (Fig.4) and based on the company's "one-step, one-button" approach, this lancing device is the first fully-automated, electronically-controlled, self-contained system that allows a patient to execute the entire lancing process at the touch of a button in a virtually painless operation. (***57***) Unlike existing mechanical lancing devices, the Pelikan Sun detects the location of the skin and penetrates to the exact depth without hitting sensitive nerves in the finger. In more details the Pelikan Sun lancet rapidly travels just to the capillary loops, nicks the capillary loops slightly, makes a soft and controlled 'pillow stop', and then slowly retracts. The lade's trajectory is controlled and does not jitter, thereby creating a straight wound channel that will not collapse. Instead, as the lancet is slowly retracted, blood follows the lancet through the smooth wound channel to the surface of the finger.

It contains a disc of 50 lancets, which is replaced after 50 uses so the person does not have to handle the lancets. The Pelikan Sun is ideal for children, frequent testers, and people who don't like the pain of lancing.

Figure 4: Pelikan Sun

1.8.6 Alternative Glucose Monitors

The home blood glucose biosensor market is dominated by four large diagnostic companies selling “finger-prick” tests which use a tiny sample of capillary blood. These companies are continually challenged by new start-ups, which are often the engines of change and the most successful, such as MediSense and TheraSense, usually end up being acquired by a larger company. The majority of the business of these companies in this market is based on single-use mediated amperometric biosensor technology. Alternative strategies include minimally invasive testing meters and continuous glucose monitoring.

1.8.6a Minimally invasive testing

The GlucoWatch G2 Biographer (***58***), (***59***) is a wrist worn device. It detects trends and tracking patterns in glucose levels in adults with diabetes, for use at home and in health care facilities. The device obtains automatic measurements of glucose concentrations every 20 minutes for up to 12 hours at a time. It is minimally invasive, since the skin is not punctured to obtain a sample. The measurements obtained by GlucoWatch are not a substitute for finger-prick blood glucose readings, since there is a 20 minute lag between the GlucoWatch monitor and the actual blood glucose value, but they can be used for the detection and assessment of episodes of hypoglycemia and hyperglycemia, facilitating both acute and long-term therapy adjustments.

The device uses reverse iontophoresis to collect glucose samples through intact skin. The glucose molecules are collected in a gel collection disk, which contains the

glucose oxidase. As glucose enters the disk it reacts with glucose oxidase in the gel in order to form hydrogen peroxide. An electrode detects the hydrogen peroxide and generates an electronic signal and by the calibration value previously entered by the patient, the signal is converted into a glucose measurement, which is displayed on the biographer and stored in the memory of the device.

The Glucowatch is produced by Cygnus (Redwood City, CA, USA) and was recently acquired by Animas, which was, in turn, acquired by Johnson and Johnson. The company does not appear to be actively promoting the device at present following some debate about its performance, but is still "supporting" the device in the market.

1.8.6b Continuous Glucose Monitoring System

Much attention has been given to the development of subcutaneously implantable needle-type electrodes, which were first described by Schichiri et al. (***60***) The sensor is presented as a fine needle or flexible wire. The active sensing element, which is placed at or near the tip is implanted in the subcutaneous tissue. Such sensors are regarded as "minimally invasive" and their subcutaneous implantation avoids the problems of sepsis, fouling with blood clot, and embolism, which are potentially associated with intravascular placement. The operational lifetime of these devices is a few days.

i) Electrochemical detection of glucose oxidase

The typical construction of these devices (Fig.5) include the glucose oxidase immobilisation at a platinum anode, an inner membrane, such as cellulose acetate, which "filters" the interfering substances and an outer membrane, such as polyurethane, that controls the diffusion of glucose and improves the

biocompatibility. The hydrogen peroxide produced by glucose oxidase is detected electrochemically. (***61***)

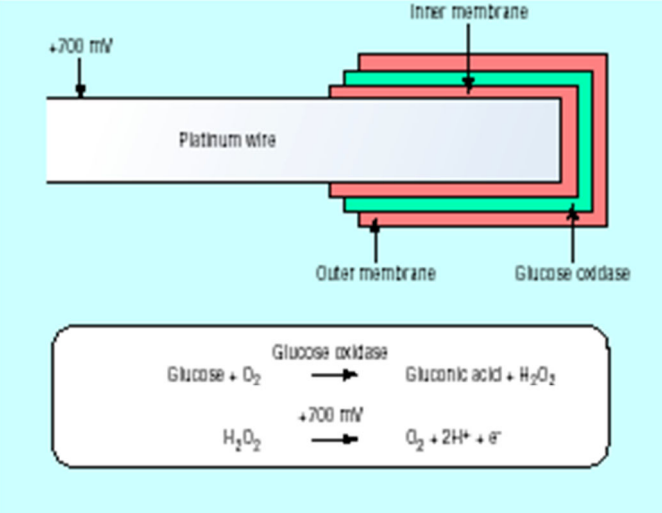


Figure 5: A typical construction of an electrochemical device to detect glucose oxidase **(***61***)**

Success in this direction has reached the level of short-term human implantation in the hands of Minimed Medtronic (Northridge, CA, USA) and Dexcom (San Diego, USA). Such devices could enable a swift and appropriate corrective action through a closed-loop insulin delivery system, for example an artificial pancreas. (***62***) Algorithms correcting for the transient difference (time lag) between blood and tissue glucose concentrations have been developed.

Medtronic is best known for its implantable devices. It traces its history back 20 years in diabetes and supports a research program for the development of a

continuous glucose sensor as well as an implantable insulin pump. The *CGMS* (Fig.6) is a sensor about the size of an AA battery that transmits radio signals to a pager-sized receiver. The device provides up to 288 glucose measurements every 24 hours, which means 72 times more data than patients testing their blood sugars four times a day would obtain. Some patients can obviously benefit from this rich source of information, while for others it may be considered simply data overload. The *CGMS System Gold* sensor can measure glucose values up to 72 hours while patients go about their normal activities. The *CGMS System Gold* sensor is typically inserted into subcutaneous tissue in the abdominal area and worn for 1 to 3 days. A Holter-style monitor stores continuous glucose data measured by the sensor at five-minute intervals. This data can be downloaded into a computer and reports can be easily printed for retrospective analysis. The system uses a needle to insert a very narrow guage enzyme electrode beneath the skin, where it encounters interstitial fluid. It is designed to be inserted into the subcutaneous tissue, usually in the abdominal area, utilising a soft cannula-type device. This diagnostic device is worn by the patient for three days and gathers and stores continuous glucose readings that can then be downloaded to a personal computer for analysis. The hypoglycemia alarm, alerts the patient when the glucose level drops below the limit established by the administering physician. (***63***)

Figure 6: CGMS System Gold sensor **(***63***)**

Another very similar approach of continuous glucose monitoring has recently been established by Dexcom and it is called *DexCom STS* Continuous Glucose Monitoring System (***62***) (Fig. 7). It is designed for a short term use, as a small insertable or implantable device. It presents the property to continuously measure the glucose levels and wirelessly to transmit all reading to a small external receiver, the *DexCom STS*. The *DexCom STS* can also alert the patient when a high or low glucose level is detected.

Figure 7: DexCom STS Continuous Glucose Monitoring System **(***62***)**

ii) Optical detection of glucose oxidase

In 2004, a novel optical glucosensor has been developed at the University of California for continuous monitoring of glucose levels. It is consisted of a fluorescent chemical complex, which is immobilised in a biocompatible polymer, permeable to glucose. The sensing system is consisted by a fluorescent dye and a quencher, which binds to the dye and prevents the fluorescence in the absence of glucose, while in the presence of glucose the quencher leads to dissociations of the complex and the fluorescence increase.

The technology has been applied in a marketable product, called *GluCath*. *GluCath* is a catheter device, used to monitoring blood glucose levels in hospitalised patients. It is inserted into a blood vessel and gives a continuous reading and can sound an alarm if the glucose level goes too high or too low. Glucose modulates the fluorescent signal by binding reversibly to a boronic acid component attached to the quencher molecule. The fluorescence is stimulated by light from an LED and can be easily measured because it occurs at a distinct wavelength from the LED light (***64***)

A long term, implantable, optical sensor, which designed to provide continuous and accurate blood glucose levels, was developed by Animas in 1997. At present, the device has been tested over a course of at least two weeks on an *in vivo* basis in dogs. The specific glucosensor is equipped with alarms in order to warn of high and low glucose levels and the final goal is the incorporation of an insulin infusion pump to provide closed loop control of blood glucose levels.

The Animas sensor (***65***) measures the near-infrared absorption of blood and is based on spectroscopy. A small display unit about the size of a wrist watch is worn on the wrist and the implanted sensor across a vein about the size of a pager transmits the reading via radio waves.

The company is in the progress of designing a miniaturised sensor, which is currently undergoing preliminary human trials. (***66***)

1.9 CHALLENGES AND HURDLES FACING GLUCOSE BIOSENSORS

Over the past forty years there has been intense activity and tremendous progress in the development of electrochemical glucose biosensors. Major advances have been made to enhance functionality of glucose measuring devices. Despite the impressive advances in glucose biosensors, there are still many challenges related to the achievement of tight, stable and reliable glycaemic monitoring. Desirable features of a biosensor system are accuracy, reliability, ultra sensitivity, fast response and low cost per test. It is very important that the biosensor system is user-friendly and understood by the patient, thus reducing incorrect results due to human error. This is

one of the reasons that the major companies dealing with glucose monitoring, are driving towards the creation of integrated devices.

In US patent 6,497,845 (***66***) issued on behalf of Roche, in December of 2002, an invention is described including a storage container for holding analytical devices (an integrated device). This invention became a commercial product after a couple of years with the launch of *Accu-Check Compact Plus* (***67***). This device is a blood glucose monitoring system based on reflectometric technology. The system consists of a meter and dry reagent test strips designed for capillary blood glucose testing by people with diabetes or by health care professionals. *Compact Plus* uses drums with 17 test strips, and the meter is automatically calibrated by inserting a new drum. An electronic check is performed automatically and a test strip is pushed forward when the meter is turned on with a button. The system requires a blood volume of 1.5 μL and provides a result within 5 seconds. The test principle of Compact Plus relies on the reaction of glucose oxidase with pyrroloquinolone quinone (PQQ). An indicator changes from yellow to blue by means of a mediator and a redox-process. The blue colour is read reflectometrically. The meter has the capacity to store 300 results in memory. Accu-Chek Softclix Plus lancet pen is fastened to the Compact Plus meter. The lancet pen can be used either when fastened to the meter or it can be taken off the meter.

A method and apparatus for handling multiple sensors in a glucose monitoring instrument system is described in US patent 5,510,266 (***68***) issued in April of 1996, in favour of Bayer. The invention is generally related to a glucose monitoring system

and, more particularly, to an improved device for handling multiple sensors that are used in analysing blood glucose. This invention became a reality in 2003, with the launch of *Ascenia Dex*, (***69***) which was the first blood glucose monitor to store multiple strips inside the meter. This device was very easy to use and its integration reduced incorrect results due to the human error. The integration of the test strips in the device was a major innovation, resulting in significant profit to the companies. This one of the reasons that these patents became for a subject of litigation between Roche and Bayer, since there is an apparent similarity between the drum and the disk that *Accu-Check Compact* and *Ascensia Dex* have.

Another challenge that glucose biosensors manufacturers had to face is the many manual operating steps in conventional lancet systems (lancet and lancing device), which is obviously disadvantageous to the user. In most of the systems that are available at present, the lancets for use in lancing devices are provided in a loose form and for each lancing process, the user manually removes a lancet from a pack and has to insert it into the lancet holder of the lancing device and fix it there. Numerous attempts to eliminate the above disadvantage have been described. In US patent 6,616,616, (***70***) issued in September 2003, Roche describes an invention concerning a lancet system comprising a plurality of essentially needle-shaped lancets, a drive unit which has a drive element in order to move the lancet from the resting position into the lancing position, a storage area to store the lancets, a withdrawal area to guide at least the tip of the lancet out of the system during the lancing process and a transport unit which can transport lancets from the storage area into the withdrawal area. The above invention became a reality in 2004, with the launch of the MultiClix (***71***). MultiClix is now one of the most popular lancing devices.

It is the only one with a six-lancet drum, combining safety and convenience, since no handling of lancets is necessary. It also provides 11 penetration depth settings, letting the patient adjusting the penetration according to his skin type, reducing by this way the pain by avoiding contact with deeper nerves.

If one follows this evolution, then the next logical product is multiple sensors combined with multiple lancets as announced by Pelikan Technologies Inc. This presents many technical challenges including achieving sufficiently high density of sensing/catalytic sites. While electrochemical technology has continued to dominate this field, one paper by the Pelikan team has explored optical solutions. (***72***) This paper describes a new type of fluorescence-optical glucose biosensor. The membrane consisted of an emulsion that incorporated the enzyme glucose oxidase capable of catalytically consuming sample glucose and reducing oxygen concentration. The emulsion additionally contained an oxygen-quenchable fluorescent indicator that produced a signal proportional to the glucose concentration in the sample. The particular advantage was seen as a high density array sensor for glucose that could be fabricated within the stringent cost restraints associated with this market.

1.10 SUMMARY

Diabetes is one of the leading causes of death and disability in the world. 171million people in the world are suffering from this disease and several studies have

demonstrated the likely increase of this figure to 366 million by 2030. Thus quick diagnosis and early prevention are critical for the control of the disease. Forty years have passed since Clark and Lyons proposed the concept of glucose enzyme electrodes. Since then, excellent economic prospects and fascinating potential for basic research have led to many sensor designs and detection principles for the biosensing of glucose.

The first-generation of biosensor devices relied on the use of an oxygen co-substrate, and the production and detection of hydrogen peroxide. Extensive efforts during the 1980s were devoted to minimising the error associated with electroactive interferences in glucose electrodes. Particularly useful has been the use of artificial mediators at the second generation of glucose biosensors. Nowadays commercial blood glucose meters are produced by many companies worldwide, but the major players are Roche Diagnostics, LifeScan, Abbott and Bayer mostly employ mediated amperometric biosensor technology. Some other alternative strategies include minimally invasive testing meters and continuous glucose monitoring. As this field enters the fifth decade of intense research, the market size and the huge demand is creating the need for the development of new approaches, like painless, *in vitro* devices, non-invasive monitoring and miniaturised long term implants with advanced biocompatible membranes. Opportunities for new technology abound and novel optical approaches continue to be published. The rigorous demands of commercialisation have so far selected in favour of electrochemical devices, but the trend towards high density arrays and multi-analytes may yet tip the balance in favour of optical techniques such as fluorescence. These and similar developments

are expected to greatly improve the control and management of diabetes to the overall benefit of the World's population.

1.11 AIMS & OBJECTIVES

Diabetes is one of the leading causes of death and disability in the world. As this field enters the fifth decade of intense research, the market size and the huge demand are creating the need for the development of new approaches. Major advances have been made to enhance functionality of glucose measuring devices. Despite the impressive advances in glucose biosensors, there are still many challenges related to the achievement of tight, stable and reliable glycaemic monitoring. Desirable features of a biosensor system are accuracy, reliability, ultra sensitivity, fast response and low cost per test.

This project was aimed at better understanding and improve the performance of electrochemical glucose biosensors.

- *In general, high surface area electrodes are desired as the high surface area provides more active sites for electrochemical reactions, and hence higher kinetic rate capability. Therefore, the determination of the active electrochemical surface area of the electrode is very important. A study has been conducted to determine the real electrochemical surface area of the Pelikan SPEs and a method has been optimised and established by Pelikan for the evaluation of their SPEs.*
- *The haematocrit differences observed in the blood samples can significantly affect glucose measurements. Therefore a study has been conducted in order*

to observe the absorption of the blood samples into the working electrode paste according to the haematocrit level

- *A new bi-functional conducting polymer N-(N, N'-diethyldicarbamoyl ethyl amido ethyl) aniline (NDDEAEA), incorporating both aniline and dithiocarbamate ester groups, which can potentially be used in the construction of novel Pelikan electrodes with enhanced integration functionalities has been fully characterised and acted as a matrix for the development of an amperometric glucose biosensor. This material is capable of grafting protective anti-adhensive layers to assure that the poor performance in sensors as a result of impact of blood components can be mitigated.*
- *The purpose of this study was to develop and optimise the reaction conditions to grafting a hyperbranched polymer onto the surface of the multi-walled carbon nanotubes (MWCNT), using the A_3 + B_2 approach. By this study we aimed to explore a further advanced approach to significantly increase the sensitivity of Pelikan SPEs.*

2ND CHAPTER

MATERIALS & METHODS

2.1 PELIKAN SUN: A COMMERCIAL AMPEROMETRIC GLUCOSE OXIDASE BIOSENSOR

2.1.1 MATERIALS

Chemicals:

The TMPD (N,N,N',N'-Tetramethyl-p-phenylenediamine, CAS No:100-22-1), was purchased from Acros-Organics (Geel, Belgium). The 2-(1-Methoxy)propyl Acetate 97%, used as diluter for preparing stock solutions of TMPD, was purchased from Acros-Organics (Geel, Belgium). All the reagents used to make the buffers (Phosphate Buffer Saline) were purchase from Sigma-Aldrich (Dorset-UK). Deionised water was used for all solution preparations. All solutions used to perform the electrochemical experiments were freshly prepared and degassed by bubbling pure nitrogen. The blood samples were purchased by Sera laboratories International and stored at 4^{o} C. The working electrode paste was supplied from Pelikan Technologies GmbH (Munster, Germany). The glass microscope slides used to deposit the working electrode paste were purchased from VWR International LTD (Lutterworth, UK).

Electrodes:

The cyclic voltammograms and the chronocoulometric plots were obtained by using either a 10mL three-electrode cell, or Pelikan screen-printed electrodes. The three-electrode cell includes a glassy carbon working electrode (1.6 mm diameter), a platinum wire counter electrode and a Ag/AgCl reference electrode, purchased from BASi Electrochemistry Instruments (Warwickshire, UK). The Acheson and Dupont screen-printed electrodes, as well as the Pelikan screen-printed electrodes were

supplied by Pelikan Technologies GmbH (Munster, Germany). The geometrical surface area of all of those screen printed electrodes is 0.25mm^2.

Apparatus:

All of the electrochemical experiments (cyclic voltammetry and chronocoulometry) were performed using either a μ-Autolab type III potentiostat (Eco Chemie, Netherlands) with the GPES software, or a PG580 potentiostat-galvanostat (Uniscan, UK) with the UiEChem™ software. All data were transferred and analysed by Microsoft Office Excel, on a personal computer. The final data included in this report are the mean of the measurements in triplicate. In order to perform the experiment with the contact angle goniometer the CAM 100 series (KSV Instruments, Finland) contact angle goniometer was used.

2.1.2 METHODS

i) Cyclic voltammetry (CV)

Cyclic voltammetry (***73***) falls into a class of potentiodynamic experimental methods. Due to the increasing development of computer controlled equipment with automated data collection and due to the development of mathematical description of potentiodynamic curves these experimental methods have expanded into laboratory practise widely in the last few decades. Nowadays these techniques enable the user to obtain the basic characteristics of the studied system regarding mainly a mechanism of electrode reactions and their kinetic parameters in relatively short period of time.

Cyclic voltammetry is characterised by smooth increase of a working electrode potential from one potential limit to the other and back. It follows that the potential limits and the potential sweep rate are the basic adjustable parameters. Also the properties of an electrolyte, mainly the concentration of electroactive species and temperature, could be affected.

The system response is then named polarisation (Figure 8) or dependence of current flowing through an electrode on its potential. This curve is sometimes also assigned as an electrochemical spectrum of the studied system.

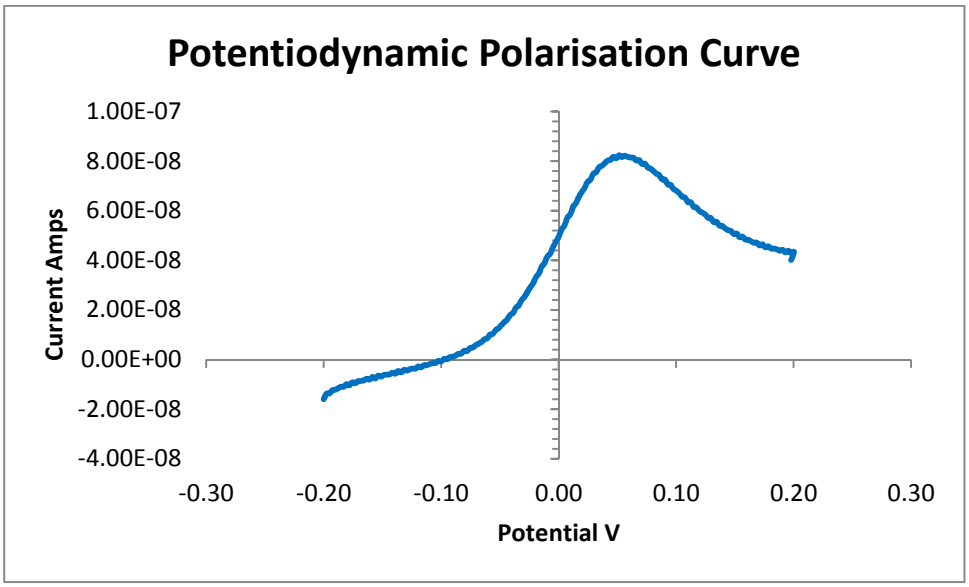


Figure 8 Potentiodynamic Polarisation Curve

Generally two limiting cases of studied systems do exist, the reversible electrode process and the irreversible electrode process.

Reversible electrode reactions:

One current peak on the potentiodynamic polarisation curve corresponds to every electrode reaction. If the equilibrium potentials of these reactions are close to each other the peaks corresponding to these reactions could overlap. Each peak could be characterised by several basic data. The potential (E_p) and current (I_p) of the peak, a half-wave potential ($E_{1/2}$ when $I=I_d/2$) and a potential at the half of the peak ($E_p/2$ when $I=I_p/2$). (Figure 9)

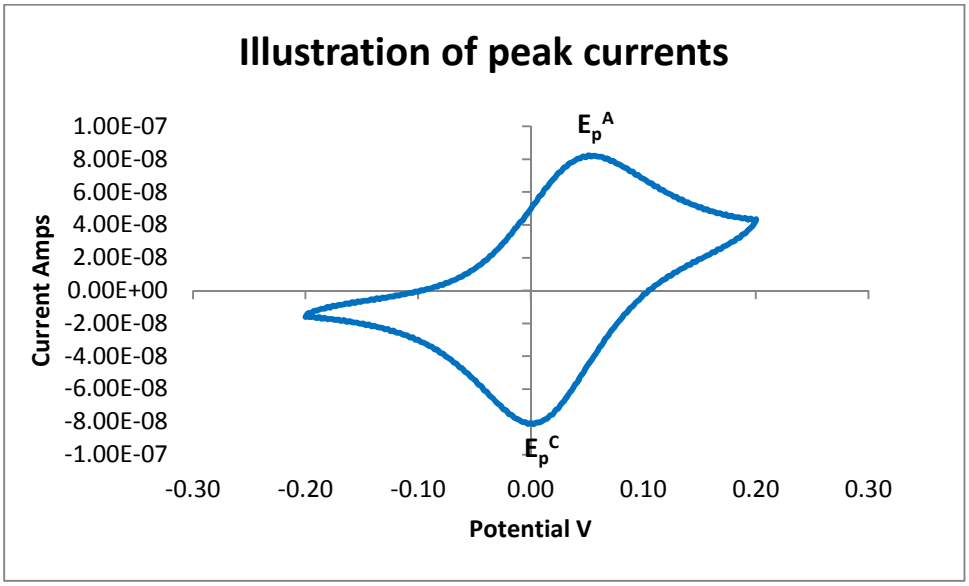


Figure 9 Illustration of the current density of a peak

For a reversible electrode reaction the surface concentration of electroactive species in every point of polarisation curve must correspond to the Nernst equation (equation 1)

$$E = E^0 + \frac{RT}{nF} ln \frac{C_o}{C_r} \qquad \text{(Eq 1)}$$

Where:

E: electrode potential
R: universal gas constant
T: temperature
F: Faraday's constant
n: number of electrons exchanged within electrode reaction
C_o: molar concentration of oxidised species
C_r: molar concentration of reduced species

Meanwhile the dependence of electrode potential on time could be presented in the case of potentiodynamic methods by the equation (2):

$$E_\tau = E_1 - v\tau \qquad \text{(Eq 2)}$$

By substituting E in Nernst equation a dependence of ratio of surface concentration of oxidized and reduced species on time and on potential sweep rate could be expressed:

$$\frac{c_{o,\tau}}{c_{r,\tau}} = exp\left[\frac{nF}{RT}\cdot\left(E_1 - v\tau - E^{0\prime}\right)\right] \qquad \text{(Eq 3)}$$

Thus in a general potentiodynamic polarisation, the peak current (Ip) could be obtained from the following equation:

$$i_p = 0{,}4463nFc_o^*\left(\frac{nF}{RT}\right)^{\frac{1}{2}} v^{\frac{1}{2}} D_o^{\frac{1}{2}}$$ (Eq 4)

Where:

I: current
n: number of electrons exchanged within electrode reaction
F: Faraday's constant
C_o: molar concentration of oxidised species
R: universal gas constant
T: temperature
v: potential sweep rate
D_o: diffusion coefficient of oxidised species

Since the above equation only reflects the value of half-peak potential, the difference between a peak potential and half-peak potential are govern by the below equations:

$$E_{p/2} = E_{1/2} + 1{,}09 \cdot \frac{RT}{nF}$$

$$\left|E_p - E_{p/2}\right| = 2{,}2 \cdot \frac{RT}{nF}$$ (Eqs 5 & 6)

It is worth noting the peak potential and other potential characteristics are sweep-rate independent and the peak current density is directly proportional to $v^{1/2}$, when the reaction is reversible. Hence these equations can be used to determine the

number of electrons exchanged within the electrode reaction or for the determination of diffusion coefficients of electroactive species. (***73***)

Irreversible electrode reactions

In comparison for a fully reversible electrode reaction the boundary condition on the surface of an electrode is determined by the kinetics of electrode raction rather than the Nernst equilibrium:

$$\frac{i}{nFA} = D_o\left[\frac{dc_{o(x,\tau)}}{dx}\right]_{x=0} = k_{(\tau)}c_{o(0,\tau)} \qquad \text{(Eq 7)}$$

Where:

I: current density
n: number of electrons exchanged within electrode reaction
F: Faraday's constant
A: surface area of the electrode
D: diffusion coefficient
d: diffusion limited variable (Id diffusion current)
k: kinetic constant

The value of its kinetic constant is given by:

$$k_{(\tau)} = k^0 \cdot exp\left\{-\alpha\frac{nF}{RT}\left[E_\tau - E^{0\prime}\right]\right\} \qquad \text{(Eq 8)}$$

By integrating the above two equations the peak current density could be described by:

$$i_p = 0{,}4958nFc_o^*\left(\frac{\alpha nF}{RT}\right)^{\frac{1}{2}} D_o^{\frac{1}{2}} v^{\frac{1}{2}} \qquad \text{(Eq 9)}$$

Since the above equation only reflects the value of half-peak potential ($E_{1/2}$), the value of a whole peak potential ($E_{p/}$) and the distance between E_p and $E_{1/2}$ could be calculated by using the following equations, respectively

$$E_p = E^{0\prime} - \frac{RT}{\alpha nF} \cdot \left[0{,}780 + ln\left(\frac{D_o^{\frac{1}{2}}}{k^0}\right) + ln\left(\frac{\alpha nFv}{RT}\right)^{\frac{1}{2}}\right]$$

$$\left|E_p - E_{p/2}\right| = \frac{1{,}857RT}{\alpha nF} \qquad \text{(Eqs 10 \& 11)}$$

On the basis of these equations the peak current density is directly proportional to the square root of potential sweep rate in the case of irreversible electrode reaction. The fact that the peak potential is a function of potential sweep rate, specifically it is directly proportional to its square root, represents, however, a significant difference from reversible process.

Therefore the cyclic voltammograms provide a useful platform for the characterization and mechanistic studies of redox reactions at electrodes.

ii) Chronocoulometry (CC)

Chronocoulometry is another electrochemical technique frequently used in electroanalytical chemistry (***74***). It is the measurement of charge as a function of time. Applications of this technique include measurement of electrode surface area, diffusion coefficients, the time window of an electrochemical cell, adsorption of electroactive species and the mechanisms and rate constants for chemical reactions coupled to electron transfer reaction. The effects of changes in potential used in the CC experiment can be understood by considering the Nernst Equation which relates the applied potential, the formal redox potential (E^O) and the surface concentrations (C^S) of the relevant electroactive species. For the redox couple $O+ne^-=R$, the Nernst Equation is:

$$E = E^0 + \frac{RT}{nF} ln \frac{C_o}{C_r} \qquad \text{(Eq 12)}$$

Where:

n = number of electrons transferred

The analysis of the chronocoulometric data is based on the Anson equation, which defines the charge-time dependence for linear diffusion control:

$$Q = 2nFACD^{½}\pi^{-½}t^{½} \qquad \text{(Eq 13)}$$

Where:

Q: charge (coulombs)

n: number of electrons transferred

A: real electrochemical surface area of the electrode (cm^2)

F: Faraday's constant (96,485 coulombs/mole)

C: the concentration of the mediator

D: diffusion coefficient of the mediator (cm^2/sec)

t: time (sec)

A plot of Q vs. $t^{½}$ is often referred as the Anson plot (***75***) The Anson plot transforms the data into a linear relationship whose slope (a) is equal to:

$2nAFCD^{1/2}/\pi^{1/2}$ (Eq. 14)

By using the above equation the real electrochemical surface area of the electrode is thus able to be determined.

iii) Standard addition method

Standard addition method is an analytical method which relies on the fact that the analytical signal is proportional to the analyte concentration. In our case it is well known that the electrical current of an electrochemical process is proportional to the concentration of the analyte used. The stadard addition equation is:

$C_X = [(A_X C_S V_S) / V_X] / [A_{X+S}((V_X+V_S)/V_X) - A_X]$ (Eq. 15)

Where:

C_x: standard concentration of the unknown

A_x: analytical signal of the unknown

C_s: standard concentration

V_s: standard volume

V_x: volume of the unknown

A_{x+s}: analytical signal of unknown plus standard

iv) Methodology followed for determing the real electrochemical surface area of the electrodes

The determination of the real surface area of the working electrode is mainly based on the use of Chronocoulometry (Figure 10). (A detailed SOP-Standard Operational Procedure- can be found in the Appendix) It is the measurement of charge as a function of time. The analysis of the chronocoulometric data is based on the Anson equation, which defines the charge-time dependence for linear diffusion control:

$Q = 2nFACD^{½}\pi^{-½}t^{-½}$

A plot of Q vs. $t^{½}$ is often referred as the Anson plot which transforms the data into a linear relationship whose slope (a) is equal to the following equation:

$a = 2nAFCD^{1/2}/\pi^{1/2}$

Where:

n: number of electrons transferred
A: real electrochemical surface area of the electrode (cm^2)
F: Faraday's constant (96,485 coulombs/mole)
C: the concentration of the mediator (mol/cm^3)
D: diffusion coefficient of the mediator (cm^2/sec)

By using the above equation the real electrochemical surface area (A) of the working electrode (cm^2) can be calculated providing the concentration (C) and the diffusion

coefficient of the mediator (D) incorporated in the working electrode paste are known.

Diffusion coefficient

The diffusion coefficient of the mediator can be determined by Cyclic Voltammetry. By immersing the electrode in PBS, the cyclic voltammograms were run with three different scan rates (10mV/sec, 25mV/sec, 50mV/sec), respectively. Three oxidation peak currents (Eox) were then plotted against the square root of the scan rates. The resulting slope of the plot is equal to the diffusion coefficient of the mediator.

Concentration of the mediator

The standard addition method was applied to calculate the concentration of the mediator present in the electrodes. In our case it is well known that the electrical current of an electrochemical process is proportional to the concentration of the analyte used.

N,N,N′,N′-tetramethyl-p-phenylendiamine (TMPD) is used by Pelikan as the mediator which is incorporated in the working electrode paste. TMPD presents two well defined one-electron oxidations. The first oxidation is reversible and can be cycled indefinitely. The second oxidation is rendered irreversible by a following chemical reaction probably related to the hydrolysis of the electrochemical product. In this study we focused on the initial one electron reversible process. (***76***), (***77***) Five TMPD solutions with different concentrations in PBS were prepared and the cyclic voltammograms were obtained by using the Pelikan electrode. However, the electrochemical response attributes to both of the mediator in PBS and the mediator incorporated in the working electrode (WE) paste. In order to observe the

electrochemical response of the mediator incorporated in the WE paste, cyclic voltammograms was run in PBS only. The oxidation peak currents observed in the cyclic voltammograms were then plotted against the concentration of the added mediator. Linear regression is performed and the slope and intercept of the calibration curve are used to calculate the unknown concentration of the mediator

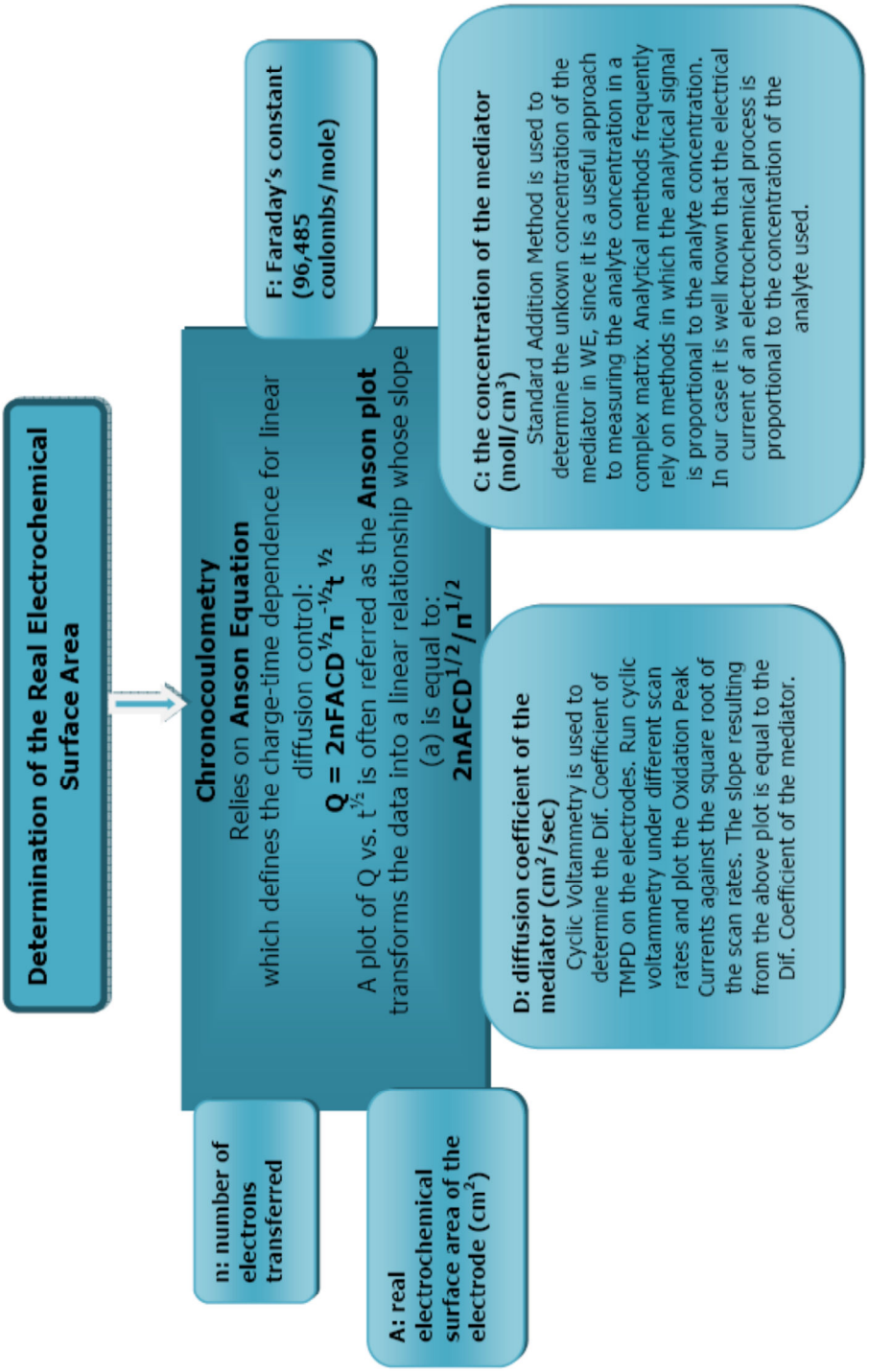


Figure 10 Schematic representation of the determination of the real electrochemical surface area

v) Haematocrit effect - Absorption of the blood sample into the working electrode paste according to the haematocrit levels

A goniometer was used in order to observe the absorption of the whole blood samples in the working electrode paste, in accordance with the heamatocrit levels. Contact angle measurements have been recorded between the WE paste and the different blood samples with different heamatocrit levels, for 16 seconds with frame intervals of 2 seconds. The heamatocrit range tested was between 25 to 40 per cent. In order to avoid the blood sample variation, just one blood sample has been used to run the experiment. The initial hematocrit level was 40% and in order to obtain blood samples with different heamatocrit levels, the initial blood sample was diluted further using physiological saline (0.9% NaCl).

2.2 AN AMPEROMETRIC GLUCOSE OXIDASE BIOSENSOR USING AN ELECTROCHEMICALLY GENERATED CONDUCTING POLYMER

2.2.1 MATERIALS

Chemicals:

All the chemicals and solvents used within this work were purchased from Sigma-Aldrich (Dorset, UK) and were of Analytical grade. The NDDEAEA was synthesized and supplied by Mrs Petya Iwanova-Mitseva. All solutions used to perform the electrochemical experiments, including the monomer solution, were freshly prepared and degassed by bubbling pure nitrogen. Glucose solutions were freshly prepared the day before each experiment in order to allow muta-rotation of the isomers and

were kept at 4^{o}C. *Glucose oxidase* from *aspergillus niger,* type II-S, 15,000-50,000 units/g solids was purchased from Sigma Aldrich (Dorset, U.K.) and was stored at -20^{o}C when not in use. The screen printed gold electrodes (SPE) (1.6 mm diameter) were purchased from Dropsens.

Apparatus:

All of the electrochemical experiments were performed using either a μ-Autolab type III potentiostat (Eco Chemie, Netherlands) with the GPES software. All data were transferred and analysed by Microsoft Office Excel, on a personal computer. The final data included in this report are the mean of the measurements in triplicate. A Philips UV lamp (8m Wcm^2) and a fiber optic light source with a 0.016 W/cm^2 intensity CERMAX® Xenon Arc Lamp (Perkin-Elmer Optoelectronics, Inc., USA) were used to irradiate the samples. Sessile water contact angle measurements were made using Cam 100 optical Angle Meter (KSV Instruments Ltd., Finland) with the software provided. Atomic Force Microscopy was performed using the DI 3000 digital instrument, while the Scanning Electron Microscopy was performed using the FEI XL300 SFEG (Scanning Field Emission Gun).

2.2.2 METHODS

i) Electrochemical Polymerisation of NDDEAEA

Before use every new electrode was cleaned and pre-treated by potential cyclic by CV from 0 to + 0.7 V, 5 times cycling, 50 mV/S scan rate in 1.5 M HCl. The stock solution of the monomer was prepared by adding 68 mg of NDDEAEA (0.2 M NDDEAEA, MW 339.52) in a 5 ml beaker in the presence of 250 μL of acetonitrile and 750 μL of 0.75 M HCl. Solution was mixed well and degassed for 10 minutes by

purging Ar/Nitrogen and covered by aluminum foil to protect from light. CV measurements were performed by placing 50 µL of the test solution of NDDEAEA onto the surface of SPE for electropolymerisation. Electrodes were cycled between -0.2 V and +0.9 V at a scan rate of 100mV /s, (step potential 7 mV) (up to 20 scans, for optimal polymer thickness). Electropolymerisation was carried out under Ar atmosphere and dark condition. Electropolymerised films were rinsed with deionised water (once), dried in a stream of nitrogen and kept dried in dark.

ii) Iniferter-activation and photocrosslinking of electropolymerised films of NDDEAEA

The electropolymerised film of NDDEAEA, formed on screen printed gold electrode, was treated with a solution of supporting electrolyte/acetonitrile/ HCl and oxygen was removed by purging pure Nitrogen for 10 minutes. The film was then irradiated for 5-15 minutes through the solution using a fibre optic UV lamp mounted at 8 cm from the sample to activate the iniferter part of NDDEAEA and generate dithiocarbamate radicals. The film was then rinsed in a stream of 50% methanol and deionised water and dried in a stream of nitrogen.

<u>Photografting of poly (methacrylic acid) to an electropolymerised film of NDDEAEA:</u>

An electropolymerised film of NDDEAEA was treated with a solution of methacrylic acid (0.1 M in acetonitrile). The solution was degassed with argon for 10 minutes before irradiation with UV for 15 minutes. The passage of argon was continued at a low flow rate during the polymerisation period. The electrode was recovered and the polymer surface was rinsed in a stream of 50% methanol and deionised water and dried in a stream of nitrogen and stored for further use.

Photografting of poly (AMPSA) to an electropolymerised film of NDDEAEA:

An electropolymerised film of NDDEAEA was immersed in a solution of AMPSA (0.1 M in acetonitrile). The solution was degassed with argon for 10 minutes before irradiation with UV for 15 minutes. The passage of argon was continued at a low flow rate during the polymerisation period. The electrode was recovered and the polymer surface was rinsed in a stream of 50% methanol and deionised water and dried in a stream of nitrogen.

Photografting of poly (Styrene) to an electropolymerised film of NDDEAEA:

An electropolymerised film of NDDEAEA was immersed in a solution of styrene (0.1 M in acetonitrile). The solution was degassed with argon for 10 minutes before irradiation with UV for 15 minutes. The passage of argon was continued at a low flow rate during the polymerisation period. The electrode was recovered and the polymer surface was rinsed in a stream of 50% methanol and deionised water and dried in a stream of nitrogen.

Layer-by-layer consecutive photografting of poly (styrene) over poly (MAA) layer grafted to an electropolymerized film of poly (NDDEAEA).

To prove the living nature of the dithiocarbamate ester groups of poly (NDDEAEA), activated during UV irradiation, consecutive layer-by-layer grafting of solutions of two different monomers, viz., MAA followed by styrene, were performed. For the layer-by-layer grafting experiment, a poly(MAA) grafted SPE electrode, prepared as described in part a) above, was treated with a solution of styrene (50 µL of 0.1 M in acetonitrile) as described in the previous part.

iii) Glucose Oxidase immobilisation

As we mentioned earlier when the poly(NDDEAEA)-modified electrode in 0.75 M HCl was irradiated with UV light, dormant DTC radicals and active carbon centred radicals, have been created. This property has been used in order to immobilise the glucose oxidase in the specific glucose biosensor. After the monomer being electropolymerised by the method described above, UV light is applied on the electropolymerised electrode, after covering it with 150 μL of GOX (30 mg/mL), for 15min. The obtained electrode was rinsed with DI water, dried carefully with Nitrogen and stored in a dark container. For calibration and characterization of glucose sensor a 5 ml of pH 7.5 phosphate buffer was placed in a container and all the measurements were carried out after the background current was stabilized, and consecutive additions of 0.1 ml of 0.5 M of glucose were added to the solution.

2.3 DEVELOPMENT AND OPTIMISATION OF THE REACTION CONDITIONS TO GRAFTING HYPERBRANCHED POLYMER ONTO THE SURFACE OF THE MULTIWALL CARBON NANOTUBES

2.3.1 MATERIALS

Chemicals:

Multi-walled carbon nanotubes (with length 20 μm, external diameter 10-20 nm and surface area> 350m^2/g) were purchased from Arry International (Germany). 1,1′-Ferrocenedicarboxaldehyde, Tris(2-aminoethyl)amine, Sodium cyanoborohydride, Thionyl chloride, Tetrahydrofuran anhydrous, ≥99.9%, inhibitor-free, triphenylphosphit and pyridine were purchased from Sigma-Aldrich (Dosret, UK).

Apparatus:

Scanning Electron Microscopy was performed using the FEI XL300 SFEG (Scanning Field Emission Gun).

2.3.2 METHODS

i) Grafting of Hyperbranched polymer onto MWCNTs using A_3 & B_2 monomers

Protocol A1 of the First Approach:

The aminated MWCNTs (10wt%) were introduced in the ultrasonic bath for 15 minutes in the presence of 100 mL of dried ethanol. The A_3 (1,1′-Ferrocenedicarboxaldehyde) and B_2 (Tris(2-aminoethyl)amine) monomers (molar ratio 1:1) were added and stirred at room temperature for 30 minutes. After they were ***refluxed for 8 hours*** at 80 ^{o}C, they were let to cool down at room temperature. The reduction step was then followed by adding a 2.5eq. of sodium borohydride. Finally the mixture was submitted to vigorous stirring for 24 hours. The product was collected by suction filtration, using a 0.22μm polycarbonate membrane and further Soxhlet extracted in tetrahydrofuran for 24 hours at 66 ^{o}C. The final product was dried in the vacuum oven at 40^{o}C for 24 hours.

Protocol A2 of the First Approach:

The aminated MWCNTs (10wt%) were introduced in the ultrasonic bath for 15 minutes in the presence of 100mL of dried ethanol. The A_3 (1,1′-Ferrocenedicarboxaldehyde) and B_2 (Tris(2-aminoethyl)amine) monomers (molar ratio 1:1) were added and stirred at room temperature for 30 minutes. After they were ***refluxed for 24 hours*** at 80 ^{o}C, they were let to cool down at room

temperature. The reduction step was followed adding a 2.5eq. of sodium borohydride. Finally the mixture was submitted to vigorous stirring for 24 hours. The product was collected by suction filtration, using a 0.22μm polycarbonate membrane and further Soxhlet extracted in tetrahydrofuran for 24 hours at 66 ^{o}C. The final product was dried in the vacuum oven at 40^{o}C for 24 hours.

Protocol B1 of the Second Approach :

The aminated MWCNTs (10wt%) were introduced in the ultrasonic bath for 15 minutes in the presence of 100mL of *N*-Methyl-2-pyrrolidon. 30mM of B_2 (Tris(2-aminoethyl)amine) monomer was mixed with two catalysts: the triphenylphosphit and the pyridine in a concentration 10 times higher than the concentration of the monomer and refluxed at 90 ^{o}C for 1 hour. After the addition of 30mM of A_3 (1,1′-Ferrocenedicarboxaldehyde) monomer and calcium chloride, in a 3 times higher concentration of the starting material, the mixture was allowed to reflux at 90 ^{o}C for ***8 hours***. The product was collected by suction filtration, using a 0.22μm polycarbonate membrane and further Soxhlet extracted in tetrahydrofuran for 24 hours at 66 ^{o}C. The final product was dried in the vacuum oven at 40^{o}C for 24 hours.

Protocol B2 of the Second Approach:

The aminated MWCNTs (10wt%) were introduced in the ultrasonic bath for 15 minutes in the presence of 100mL of N-Methyl-2-pyrrolidon. 30mM of B_2 (Tris(2-aminoethyl)amine) monomer was mixed with two catalysts: the triphenylphosphit and the pyridine in a concentration 10 times higher than the concentration of the monomer and refluxed at 90 ^{o}C for 1 hour. After the addition of 30mM of A_3 (1,1′-Ferrocenedicarboxaldehyde) monomer and calcium chloride, in a 3 times higher

concentration of the starting material, the mixture was allowed to reflux at 90 ^{o}C for ***24 hours***. The product was collected by suction filtration, using a 0.22μm polycarbonate membrane and further Soxhlet extracted in tetrahydrofuran for 24 hours at 66 ^{o}C. The final product was dried in the vacuum oven at 40^{o}C for 24 hours.

3RD CHAPTER

RESULTS

3.1 PELIKAN SUN: A COMMERCIAL AMPEROMETRIC GLUCOSE OXIDASE BIOSENSOR

3.1.1 DETERMINATION OF THE REAL ELECTROCHEMICAL SURFACE AREA OF THE ELECTRODES

The research aimed at optimisation of the performance of Pelikan sensors involved two parts: analysis of surface area of the electrodes and study of the impact of haematocrit effects on sensor performance.

i) Determination of the real electrochemical surface area of a glassy carbon electrode

As can been seen in Figure 11a, 11b and 11c the cyclic voltammograms of 0.5mM, 1mM and 2mM TMPD were recorded respectively in 0.1M Phosphate buffer, pH 7.4, containing 0.154M NaCl as a supporting electrolyte, at a 10mL three-electrode cell, with a glassy carbon working electrode, a platinum wire counter electrode and a Ag/AgCl reference electrode. The applied scan rates were 10, 25 and 50mV per second.

The resulting diffusion coefficients are presented in Figure 12 with respect to the different concentrations of the mediator (TMPD). It was observed that by increasing the concentration of the mediator in the solution, the diffusion coefficient increased.

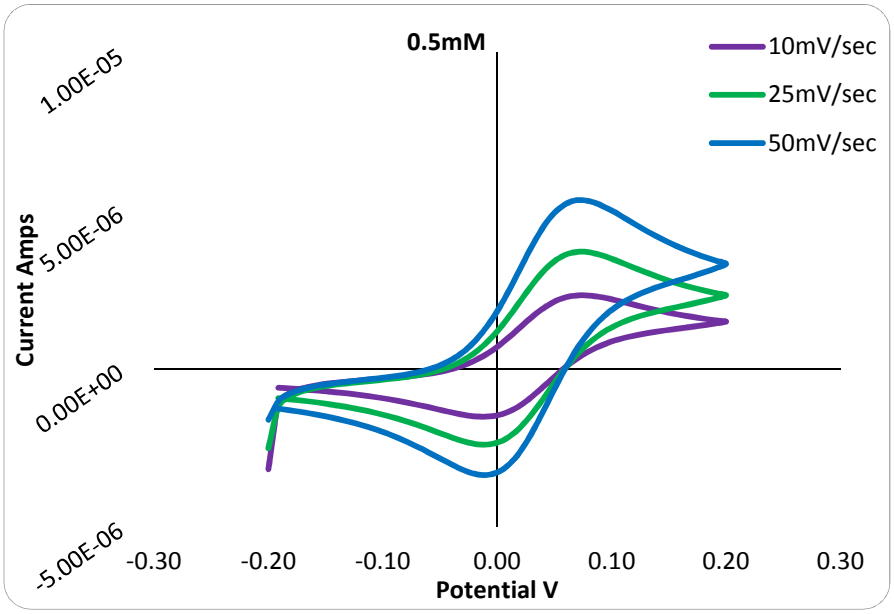


Figure 11a: Cyclic Voltammograms of 0.5mM TMPD in 0.1M Phosphate buffer, pH 7.4, containing 0.154M NaCl as a supporting electrolyte, at a 10mL three-electrode cell, with a glassy carbon working electrode, a platinum wire counter electrode and a Ag/AgCl reference electrode.

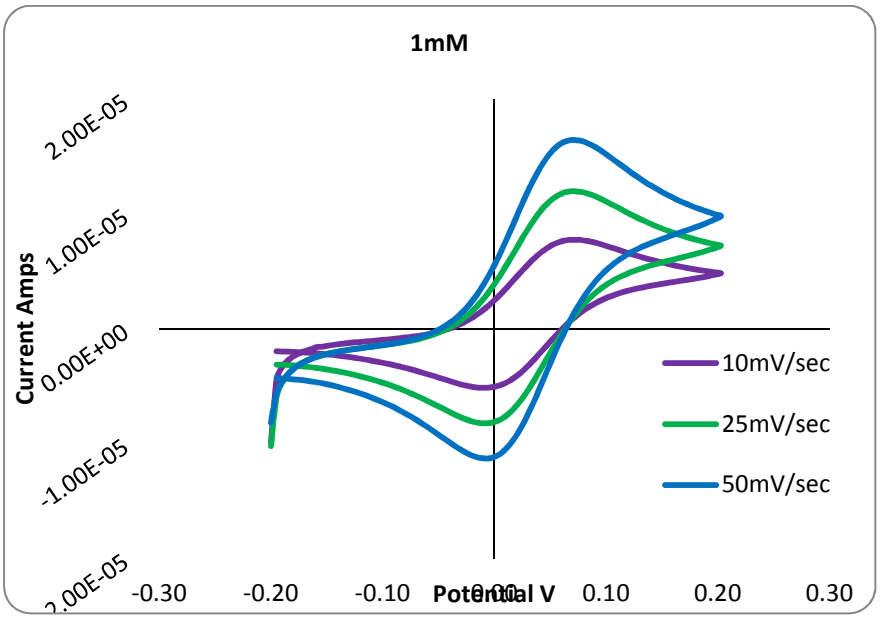


Figure 11b Cyclic Voltammograms of 1mM TMPD in 0.1M Phosphate buffer, pH 7.4, containing 0.154M NaCl as a supporting electrolyte, at a 10mL three-electrode cell, with a glassy carbon working electrode, a platinum wire counter electrode and a Ag/AgCl reference electrode.

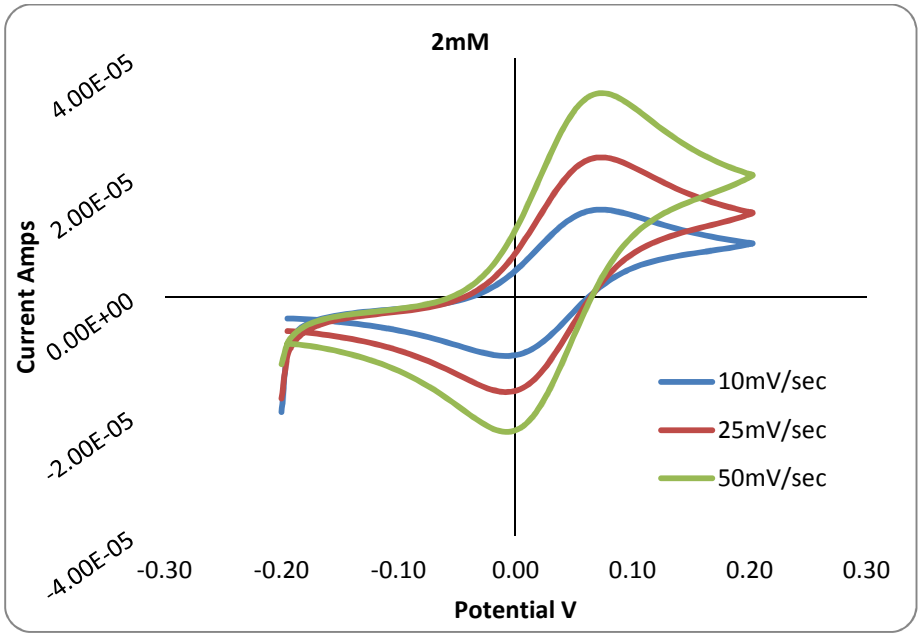
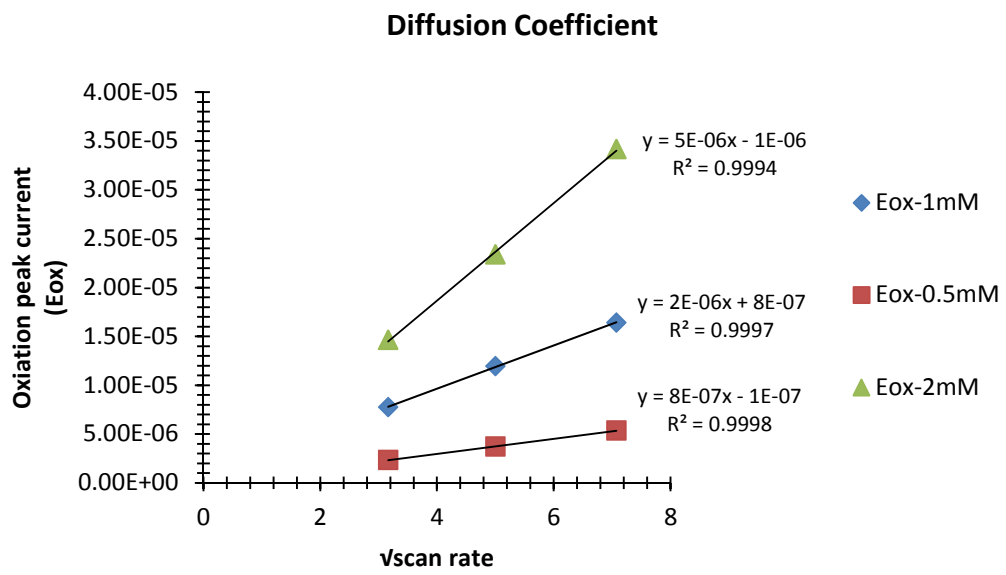


Figure 11c Cyclic Voltammograms of 2mM TMPD in 0.1M Phosphate buffer, pH 7.4, containing 0.154M NaCl as a supporting electrolyte, at a 10mL three-electrode cell, with a glassy carbon working electrode, a platinum wire counter electrode and a Ag/AgCl reference electrode.



scan rates	√scan rate	Eox (0.5mM TMPD)	Eox (1mM TMPD)	Eox (2mM TMPD)
10mV/sec	3.162	2.33E-06	7.73E-06	1.46E-05
25mV/sec	5	3.71E-06	1.20E-05	2.34E-05
50mV/sec	7.072	5.34E-06	1.64E-05	3.42E-05
D		**7.709E-07**	**2.219E-06**	**4.998E-06**

Figure 12 Diffusion coefficients (D) of the mediator (TMPD) with different concentration (0.5mM, 1mM and 2mM)

The results of chronocoulometry are presented in Figure 13. The total charge passed of 0.5, 1 and 2mM TMPD versus time were recorded using the glassy carbon electrode. The measurement was conducted after dipping the electrodes in 10mL TMPD solution. The pulse width was 2sec.

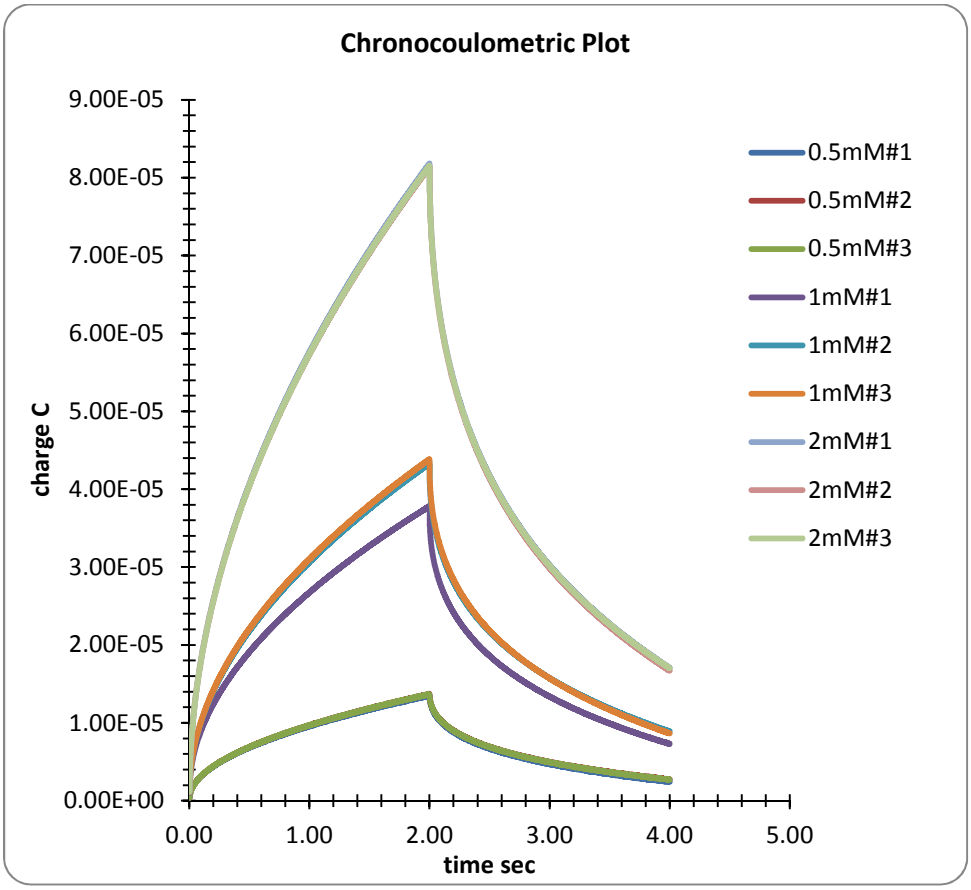


Figure 13 The chronocoulometric results of 0.5mM, 1mM and 2mM TMPD using the glassy carbon electrode **(**No of Steps: 2, E_{IN}: -0.20V, E_{FIN}: 0.2V, Duration: 4sec, Pulse Width: 2sec)

The following Anson plots are shown in Figure 14. By using the Anson equation, the real electrochemical surface area of the glassy carbon electrode was able to be calculated (Table 7) as described previously in the part of methods.

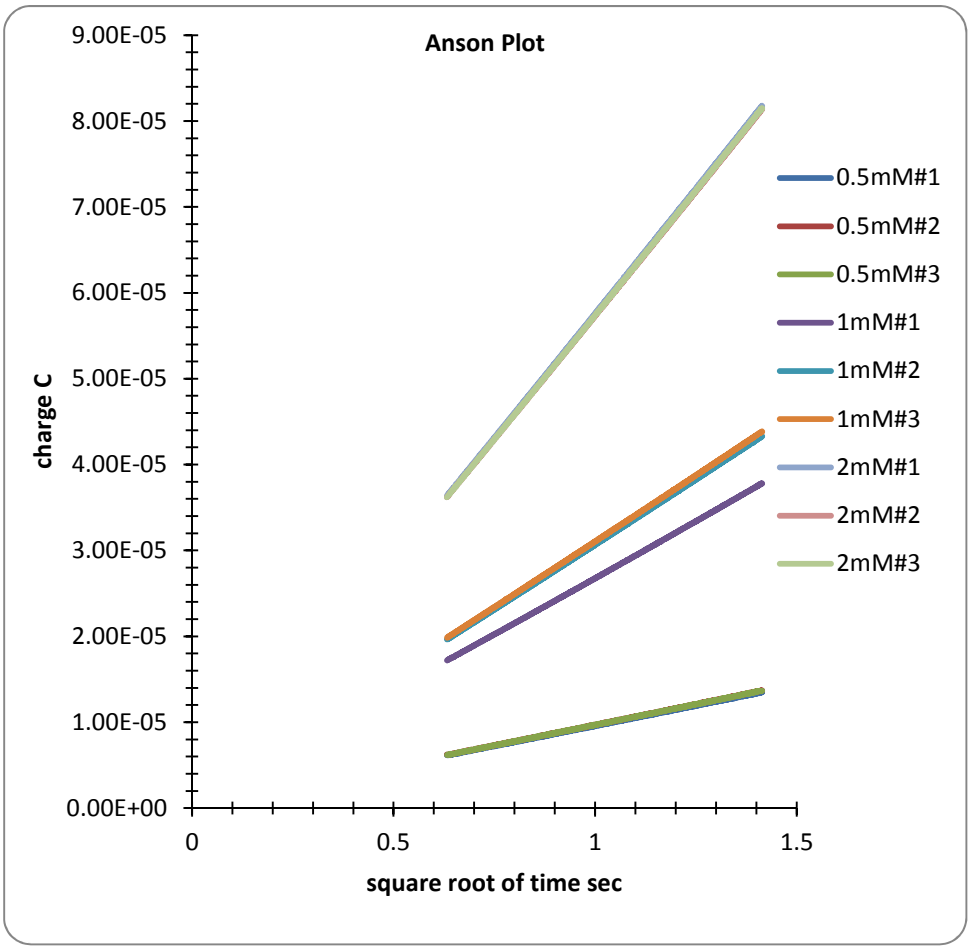


Figure 14 Anson plot – forward step (glassy carbon electrode)

It was further found that the real electrochemical surface area of the glassy carbon electrode decreased along with the increasing concentration of mediator TMPD in the solution. On the contrary, the diffusion coefficient increased by enhancing the concentration of the TMPD.

Table 7 Real electrochemical surface area of the glassy carbon electrode

REPLICATES	slope	C (mol/cm^3)	D	A (mm^2)	
0.5mM#1	9.42E-06	0.0000005	7.10E-07	20.53	
0.5mM#2	9.61E-06	0.0000005	7.10E-07	20.94	
0.5mM#3	9.59E-06	0.0000005	7.10E-07	20.90	
				20.79	**AVERAGE**
				0.22	**STDEV**
				1.09	**CV%**
1mM#1	2.64E-05	0.000001	2.22E-06	16.27	
1mM#2	3.03E-05	0.000001	2.22E-06	18.67	
1mM#3	3.00E-05	0.000001	2.22E-06	18.48	
				17.81	**AVERAGE**
				0.13	**STDEV**
				0.70	**CV%**
2mM#1	5.81E-05	0.000002	5.00E-06	11.93	
2mM#2	5.78E-05	0.000002	5.00E-06	11.86	
2mM#3	5.80E-05	0.000002	5.00E-06	11.90	
				11.90	**AVERAGE**
				0.03	**STDEV**
				0.26	**CV%**

ii) Determination of the real electrochemical surface area of Acheson and Dupont SPE.

The Acheson and Dupont electrodes present the same dimensions with the Pelikan screen printed electrodes. The only major difference is that their working electrode paste was made of carbon paste. In contrast, the working electrode paste used for Pelikan screen printed electrodes is a complex matrix containing carbon paste, enzyme (glucose oxidase), mediator (TMPD) and several buffers. The studies on Acheson and Dupont screen printed electrodes were therefore designed to generate some preliminary results excluding the effect of other elements, such as mediator and enzyme.

The cyclic voltammograms of three replicates, recorded for 100μM TMPD in 0.1M Phosphate buffer, pH 7.4, containing 0.154M NaCl as a supporting electrolyte, at the Acheson screen printed electrodes are presented in Figure 15. The scan rates applied were 10, 25 and 50mV per second.

The diffusion coefficients of the mediator using the Acheson screen printed electrodes are presented in Table 8. It was calculated that the diffusion coefficient of the mediator is 6.519E-06 cm^2/sec.

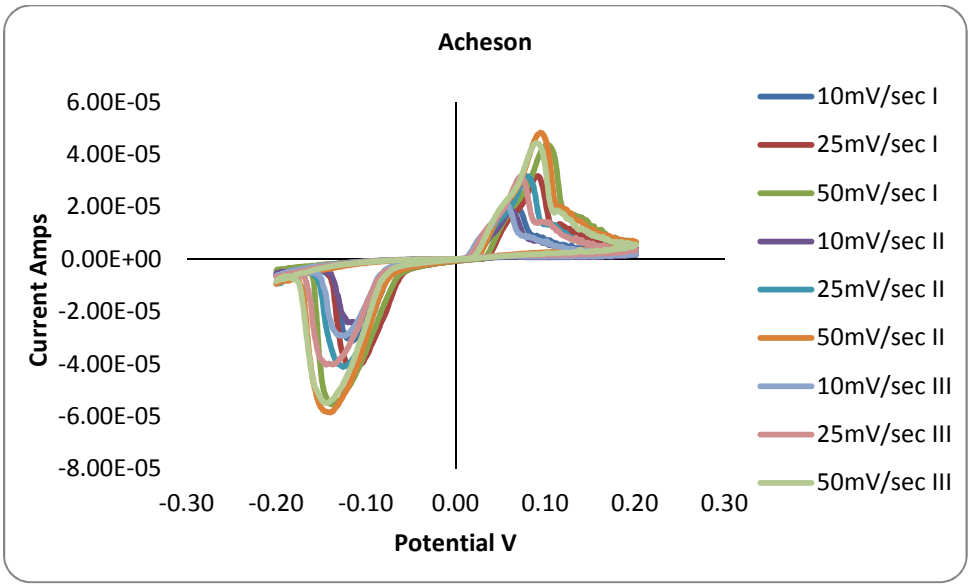


Figure 15 The cyclic voltammograms of three replicates, recorded for 100μM TMPD in 0.1M Phosphate buffer, pH 7.4, containing 0.154M NaCl as a supporting electrolyte, at the Acheson screen printed electrodes. The scan rates applied were 10, 25 and 50mV per second

Table 8 Diffusion Coefficient of TMPD on the Acheson Electrodes

Scan rates	√scan rate	Eox I	Eox II	Eox III		
10mv/sec	3.162	2.07E-05	1.86E-05	2.07E-05		
25mV/sec	5.000	3.17E-05	3.18E-05	3.15E-05		
50mV/sec	7.071	4.36E-05	4.83E-05	4.44E-05		
D		**5.867E-06**	**7.616E-06**	**6.073E-06**	**6.519E-06**	**AVR**
					9.554E-07	**STD**
					14.656	**CV %**

Figure 16 shows the chronocoulometric response, the total charge passed versus time of 100μM of TMPD recorded on the Acheson screen printed electrode. The measurement occurred after applying on the electrodes 0.7μL solution of 100μM TMPD. The pulse width was 2sec.

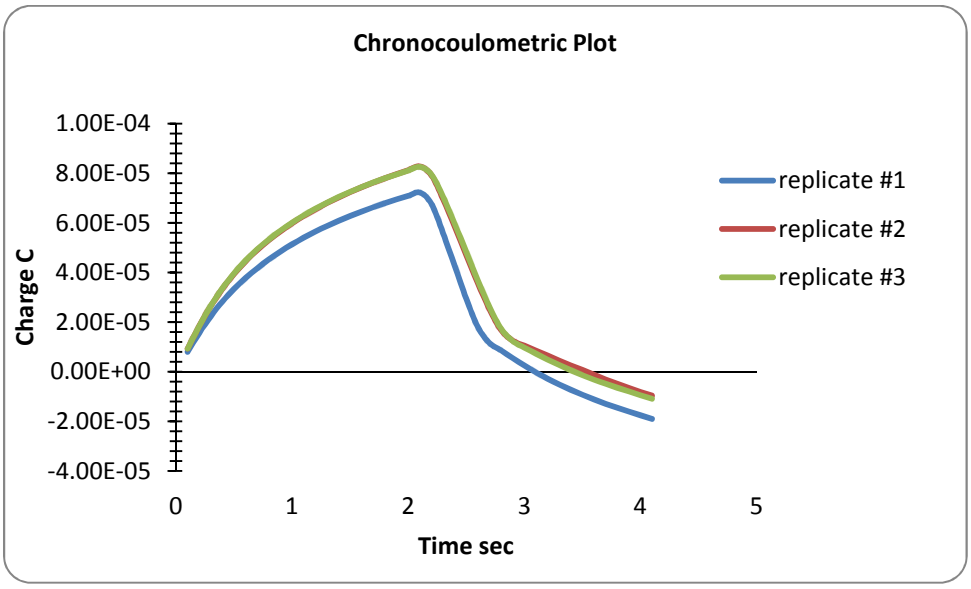


Figure 16 Chronocoulometric Plot (Acheson electrodes) **(**No of Steps: 2, E_{IN}: -0.20V, E_{FIN}: 0.2V, Duration: 4sec, Pulse Width: 2sec)

Anson plot is represented in Figure 17. By using the Anson equation described above the real electrochemical surface area of the Acheson screen printed electrode was calculated. The results are shown in table 9. The real electrochemical surface area of the Acheson screen printed electrodes is approximately 178.71 mm^2.

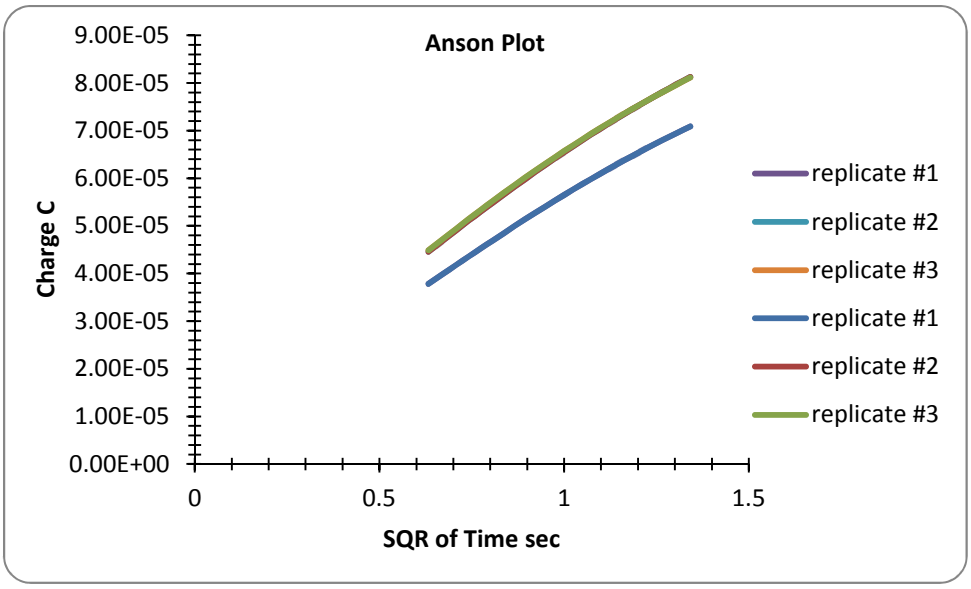


Figure 17 Anson Plot – forward step- (Acheson electrodes)

Table 9 Real electrochemical surface area of the Acheson Electrodes

REPLICATES	slope	C (mol/cm^3)	D	A (mm^2)	
#1	4.66E-05	1E-07	6.52E-06	167.525	
#2	5.16E-05	1E-07	6.52E-06	185.569	
#3	5.09E-05	1E-07	6.52E-06	183.056	
				178.71	**AVR**
				9.773	**STD**
				5.468	**CV %**

The cyclic voltammograms of three replicates, recorded for 100µM TMPD in 0.1M Phosphate buffer, pH 7.4, containing 0.154M NaCl as a supporting electrolyte, at the

Dupont screen printed electrodes are presented in Figure 18. The scan rates applied were 10, 25 and 50mV per second.

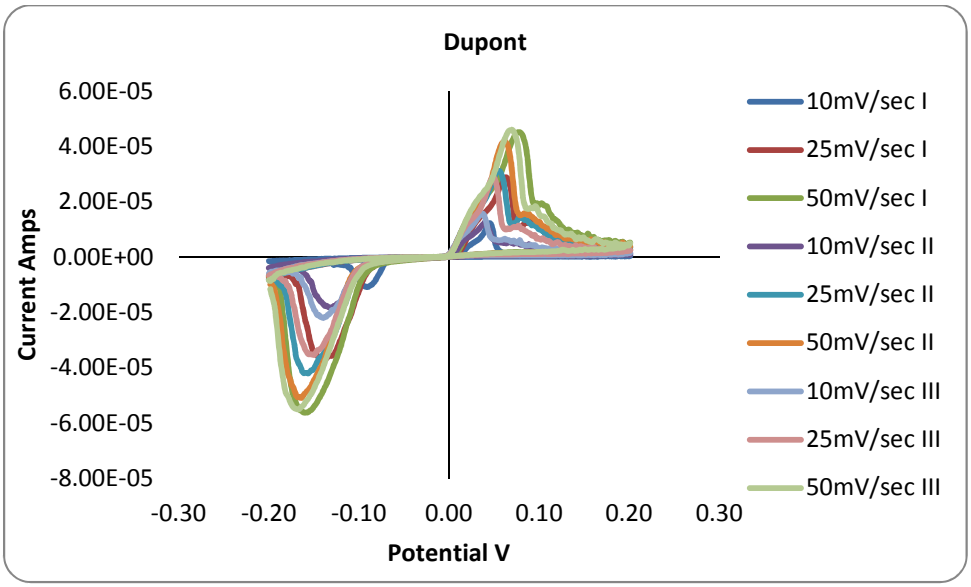


Figure 18 The cyclic voltammograms of three replicates, recorded for 100µM TMPD in 0.1M Phosphate buffer, pH 7.4, containing 0.154M NaCl as a supporting electrolyte, at the Dupont screen printed electrodes. The scan rates applied were 10, 25 and 50mV per second

The diffusion coefficient of the mediator on the Dupont screen printed electrodes is presented in table 10. It was calculated that the diffusion coefficient of the mediator is 7.859E-06 cm^2/sec.

Table 10 Diffusion Coefficient of TMPD on the Dupont Electrodes

scan rates	√scan rate	Eox I	Eox II	Eox III		
10mv/sec	3.162	1.23E-05	1.26E-05	1.58E-05		
25mV/sec	5.000	2.89E-05	3.12E-05	2.89E-05		
50mV/sec	7.071	4.52E-05	4.19E-05	4.60E-05		
D		**8.406E-06**	**7.428E-06**	**7.742E-06**	**7.859E-06**	**AVR**
					4.995E-07	**STD**
					6.356	**CV%**

Figure 19 shows the chronocoulometric response, the total charge passed versus time of 100μM of TMPD recorded on the Dupont screen printed electrode. The measurement occurred after applying on the electrodes 0.7μL solution of 100μM TMPD. The pulse width was 2sec.

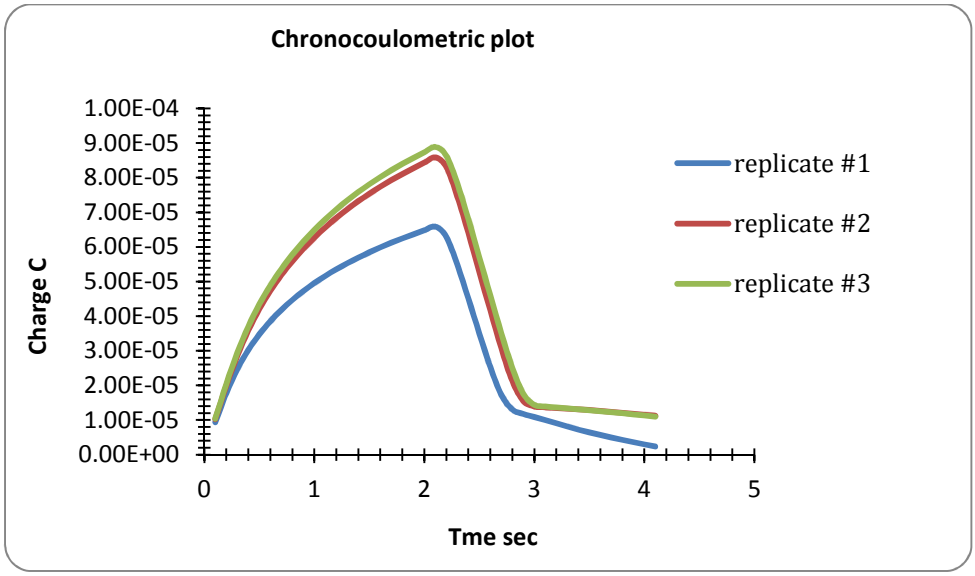


Figure 19 Chronocoulometric Plot (Dupont electrodes) **(**No of Steps: 2, E_{IN}: -0.20V, E_{FIN}: 0.2V, Duration: 4sec, Pulse Width: 2sec)

The Anson plot is represented in Figure 20. By using the Anson equation described above the real electrochemical surface area of the Dupont screen printed electrode was calculated. The results are shown in table 11. The real electrochemical surface area of the Dupont screen printed electrodes is approximately 175.87 mm^2.

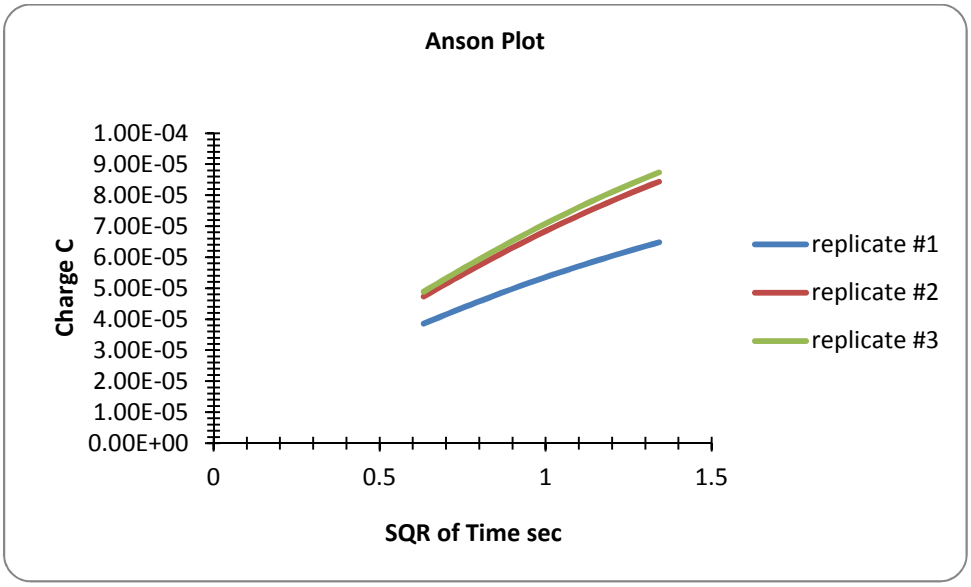


Figure 20 Anson Plot –forward step- (Dupont electrodes)

Table 11 Real electrochemical surface area of the Dupont Electrodes

REPLICATES	slope	C (mol/cm^3)	D	A (mm^2)	
#1	5.50E-05	0.0000001	7.859E-06	180.130	
#2	5.20E-05	0.0000001	7.859E-06	170.304	
#3	5.41E-05	0.0000001	7.859E-06	177.182	
				175.87	**AVR**
				5.041	**STD**
				2.866	**CV %**

The major limitation using chronocoulometry (CC) as has already been mentioned, is the fact that CC relies on the Anson equation which defines the charge time dependence for linear diffusion controlled environments. Our experiment using the Acheson and Dupont electrodes do not rely on diffusion controlled environments, since the cyclic voltammograms obtained show irreversible reactions (the separation peak currents are greater than 59mV/electron). The overall results obtained are not conclusive.

iii) Determination of the real electrochemical surface area of Pelikan SPE

Five TMPD solutions with different concentrations in PBS were prepared and used to run cyclic voltammetry. However, the electrochemical response attributes to both of the mediator in PBS and the mediator incorporated in the working electrode (WE) paste. In order to observe the electrochemical response of the mediator incorporated in the WE paste, cyclic voltammograms was also run in PBS only. The oxidation peak currents observed in the cyclic voltammograms (Figure 21) were then plotted against the concentration of the added mediator. The concentration of the TMPD can be calculated from the slope and intercept of the standard addition calibration curve. (Figure 22)

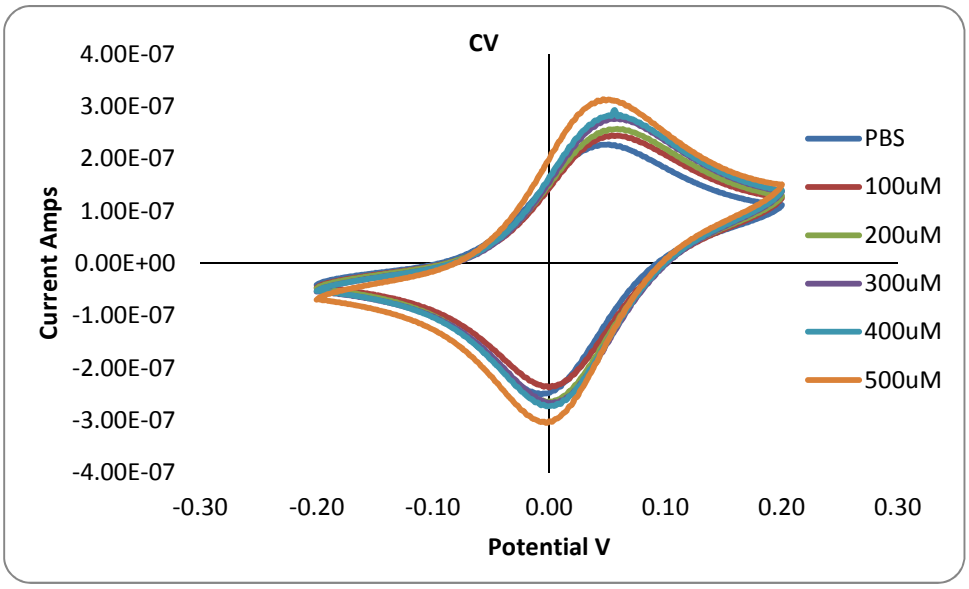


Figure 21 Cyclic Voltammograms on Pelikan electrodes

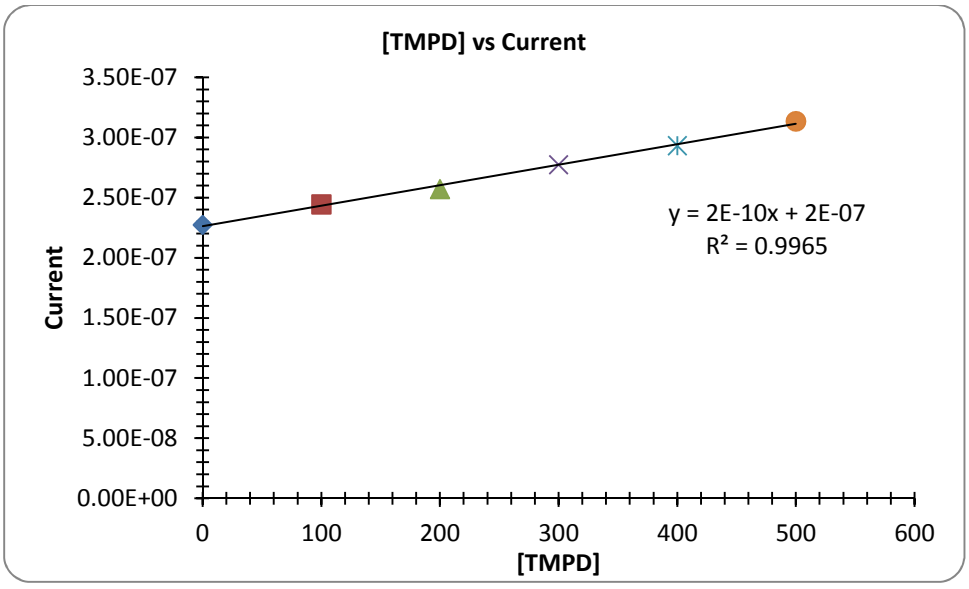


Figure 22 Standard addition calibration curve

iv) Evaluation method for pelikan SPE

There are in total 15 different batches of screen printed electrodes supplied by Pelikan. The WE paste of six of these electrodes was prepared by a ball mill. The real electrochemical surface area of these electrodes was calculated using

Chronocoulometry, after determing initially the unknown concentration of the mediator incorporated in the working electrode paste and the diffusion coefficient. The detailed results are presented on the Table 12. The concentration of the mediator incorporated in the working electrode paste ranges from 1.92mM to 4.59mM, while the diffusion coefficient ranges from 3.28e-08 cm^2/sec to 1.06e-07 cm^2/sec. By using the Anson equation described above the real electrochemical surface area of each sensor was calculated.

It was observed that there is a close relation among the concentration of the mediator, the diffusion coefficient and the real electrochemical surface area of these electrodes. As similar to the previous experiment performed applying the glassy carbon working electrode, the real electrochemical surface area of these screen printed electrodes decreased along with the increasing concentration of mediator TMPD present in the WE paste. On the contrary, the diffusion coefficient increased by enhancing the concentration of the mediator TMPD.

Table 12 Screen printed electrodes – Ball Mill

BALL MILL				
Lot No	**TMPD Concentration**	**Dif. Coefficient**	**RESA**	**CV %**
50	**4.595**	**1.06E-07**	**0.585**	**5.84**
51	**4.515**	**7.89E-08**	**0.649**	**4.67**
54	**3.027**	**5.98E-08**	**0.804**	**4.25**
57	**2.846**	**5.96E-08**	**0.848**	**9.55**
41	**1.925**	**3.28E-08**	**1.219**	**18.79**
28	**2.120**	**5.22E-08**	**1.285**	**14.30**

The real electrochemical surface area, the TMPD concentration and the diffusion coefficient of the mediator of the other nine lots of electrodes are presented in Table 13. The working electrode paste of these sensors was prepared using a three roll mill. The real electrochemical surface area of these sensors was calculated using Chronocoulometry, after determing initially the unknown concentration of the mediator incorporated in the working electrode paste and the diffusion coefficient. The concentration of the mediator incorporated in the working electrode paste ranges from 2.67mM to 4.17mM, while the diffusion coefficient ranges from 5.43e-08 cm^2/sec to 1.06e-07 cm^2/sec. By using the Anson equation the real electrochemical surface area of each sensor was calculated.

Table 13 Screen printed electrodes – Three Roll Mill

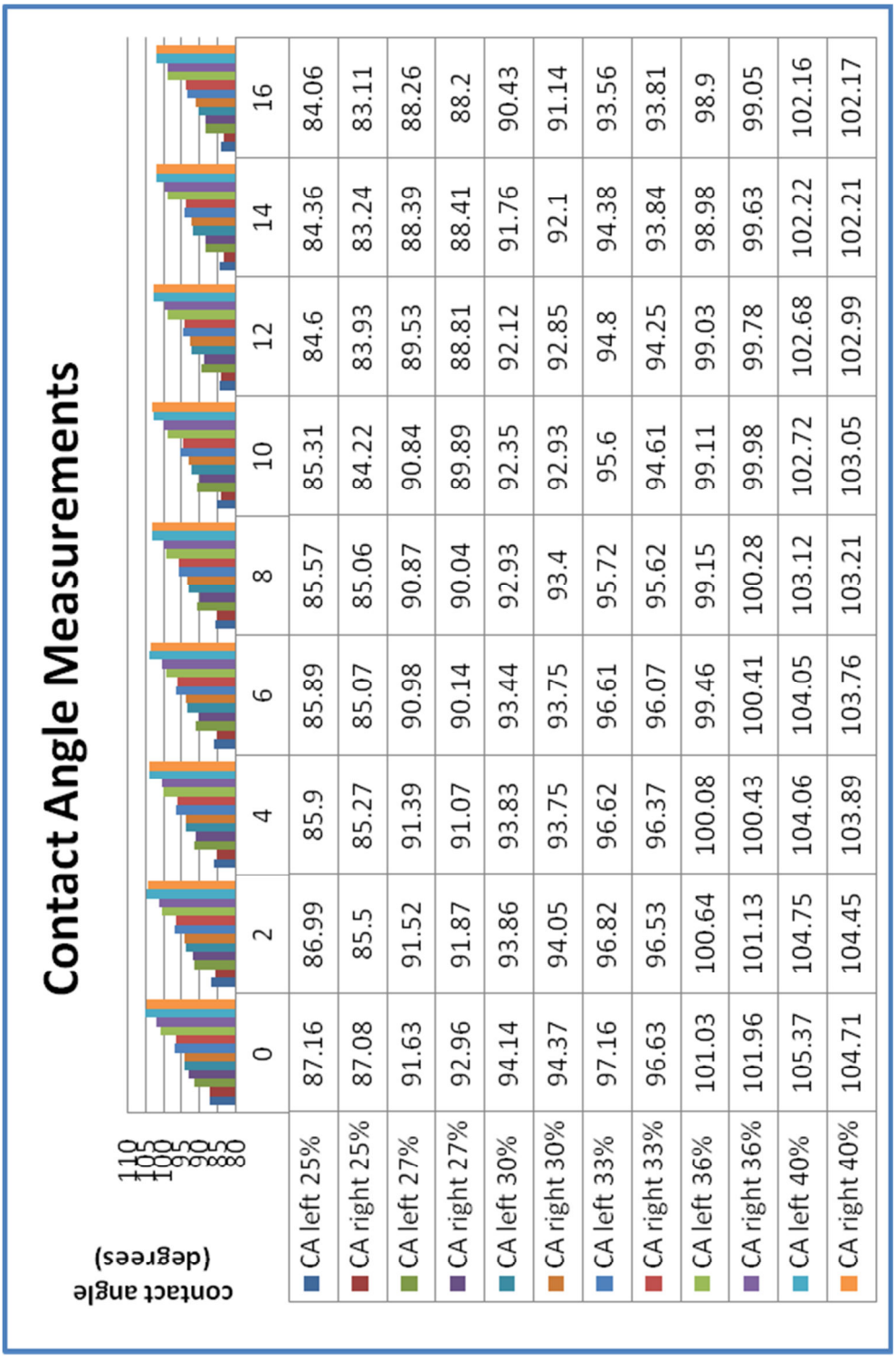
3 ROLL MILL				
Lot No	**TMPD Concentration**	**Dif. Coefficient**	**RESA**	**CV %**
114	**4.170**	**7.38E-08**	**0.550**	**4.72**
119	**3.427**	**6.87E-08**	**0.590**	**14.83**
109	**3.860**	**6.75E-08**	**0.629**	**10.08**
72	**3.033**	**5.43E-08**	**0.637**	**10.45**
71	**3.455**	**7.29E-08**	**0.727**	**8.99**
112	**3.139**	**7.80E-08**	**0.735**	**7.071**
118	**3.419**	**6.24E-08**	**0.807**	**10.44**
115	**3.250**	**6.78E-08**	**0.835**	**9.88**
91	**2.671**	**6.17E-08**	**0.853**	**8.01**

3.3.2 HAEMATOCRIT EFFECT-ADSORPTION OF THE BLOOD SAMPLE INTO THE WORKING ELECTRODE PASTE ACCORDING TO THE HAEMATOCRIT LEVELS

The hydrophobicity of a surface can be quantified by depositing a small droplet on the surface and then measuring the angle between the advancing liquid and the surface. This angle is referred as the contact angle. (***78***). In Figure 23 the contact angles obtained between the WE paste and blood samples with different haematocrit levels are shown. According to the contact angles the surface of the WE paste is

converted to more hydrophilic or hydrophobic in accordance with different haematocrit levels of each blood sample. More specifically the blood samples with haematocrit lower than 27% and consequently contact angle lower than 90^{o}, convert the surface of the WE paste to being more hydrophilic. The blood samples with heamatocrit greater than 27% and consequently contact angle greater than 90^{o}, create a hydrophobic surface.

This hydrophobicity or hydrophilicity may interfere with the adsorption of the blood on the WE paste. According to the results obtained there is a significant difference of adsorption between the blood samples with different haematocrit level, but the overall adsorption in accordance with time is not very significant. More precisely, blood samples with low haematocrit can be adsorbed easier, since the contact angle is lower and consequently the hydrophobicity of the surface of the WE paste, than blood samples with high haematocrit level.



	0	2	4	6	8	10	12	14	16
CA left 25%	87.16	86.99	85.9	85.89	85.57	85.31	84.6	84.36	84.06
CA right 25%	87.08	85.5	85.27	85.07	85.06	84.22	83.93	83.24	83.11
CA left 27%	91.63	91.52	91.39	90.98	90.87	90.84	89.53	88.39	88.26
CA right 27%	92.96	91.87	91.07	90.14	90.04	89.89	88.81	88.41	88.2
CA left 30%	94.14	93.86	93.83	93.44	92.93	92.35	92.12	91.76	90.43
CA right 30%	94.37	94.05	93.75	93.75	93.4	92.93	92.85	92.1	91.14
CA left 33%	97.16	96.82	96.62	96.61	95.72	95.6	94.8	94.38	93.56
CA right 33%	96.63	96.53	96.37	96.07	95.62	94.61	94.25	93.84	93.81
CA left 36%	101.03	100.64	100.08	99.46	99.15	99.11	99.03	98.98	98.9
CA right 36%	101.96	101.13	100.43	100.41	100.28	99.98	99.78	99.63	99.05
CA left 40%	105.37	104.75	104.06	104.05	103.12	102.72	102.68	102.22	102.16
CA right 40%	104.71	104.45	103.89	103.76	103.21	103.05	102.99	102.21	102.17

Figure 23 Contact angle measurements on blood samples (haematocrit level 25%-40%) (each measurement is the average value of 5 replicates)

3.2 AN AMPEROMETRIC GLUCOSE OXIDASE BIOSENSOR USING AN ELECTROCHEMICALLY GENERATED CONDUCTING POLYMER

The aim of this study was the characterisation of this new conducting polymer N-(N, N'-diethyldicarbamoyl ethyl amido ethyl) aniline (NDDEAEA), which can potentially be used in the construction of novel Pelikan electrodes with enhanced integration functionalities, e.g. grafting non-adhesive polymer coatings and the development of an amperometric glucose biosensor.

3.2.1 ELECTROCHEMICAL POLYMERISATION OF NDDEAEA

Figure 24 shows the electrochemical polymerisation of NDDEAEA by cyclic voltammetry. The cyclic voltammograms were obtained during deposition of 0.1M NDDEAEA (in 0.75 M HCl and 25% Acetonitrile, 3:1 solution) within the potential range of -0.2 to + 0.9 V at a scan rate of 100 mV s^{-1} for 20 potential scans, on gold screen printed electrode.

The cyclic voltammograms show an anodic peak at +0.65V and a cathodic peak at +0.52V. The large currents observed at the positive end of the cyclic voltammograms are due to the electron transfer from the poly (NDDEAEA) film corresponding to the oxidation of the PANI film and due to the electron transfer from the NDDEAEA monomer to the electrode corresponding to the oxidation of the NDDEAEA monomer to produce a precursor for the PANI film.

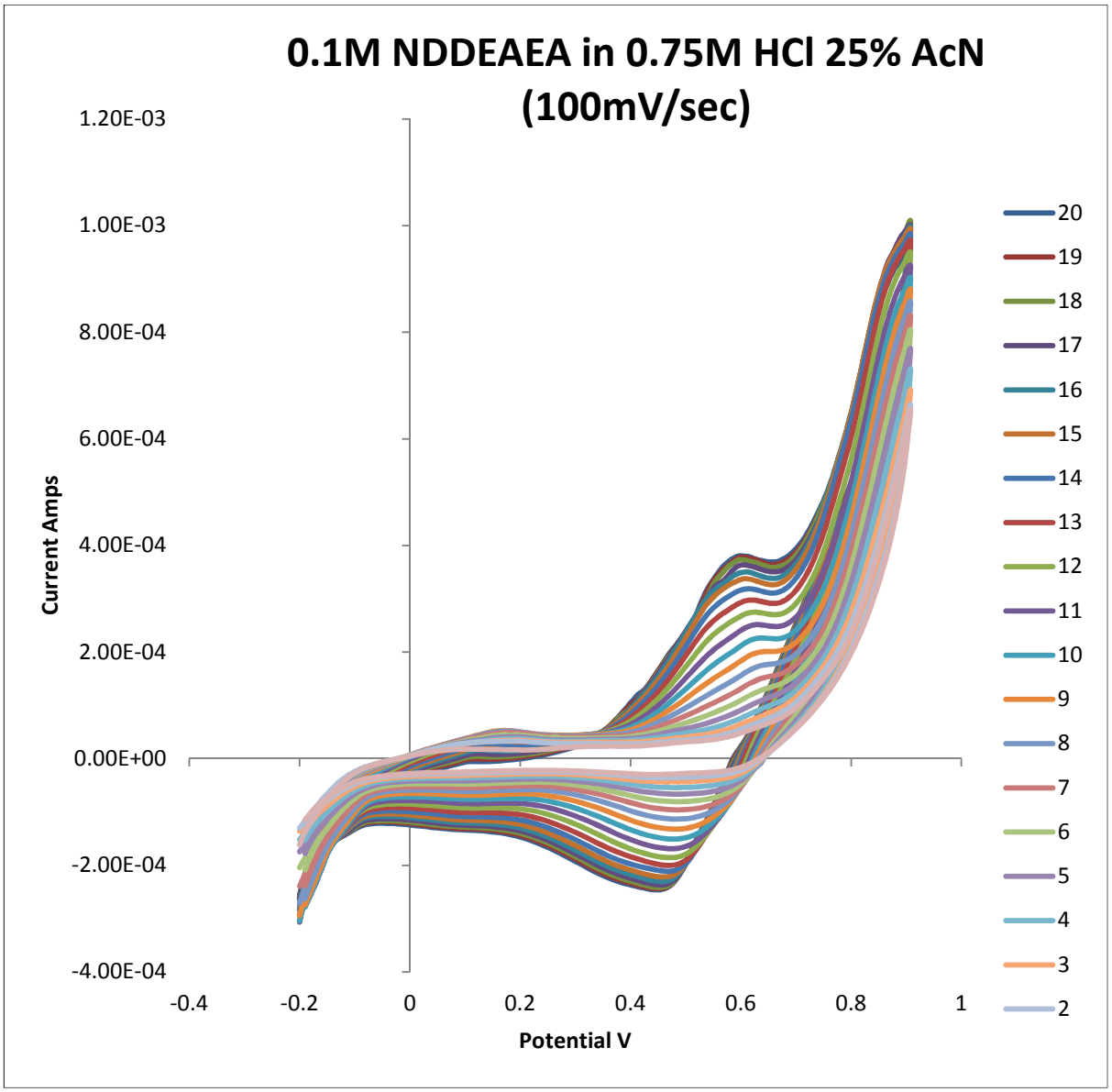


Figure 24 Cyclic voltammetric responses obtained during deposition of NDDEAEA on gold screen-printed electrode

The stability of the of poly(NDDEAEA) was investigated by dipping the electropolymerised electrodes in 0.75M HCl solution, while running cyclic voltammetry, under the same conditions of depositing 0.1M NDDEAEA on the gold

screen printed electrodes. The peaks current obtained prove the stability of the electropolymerised electrode. (Figure 25)

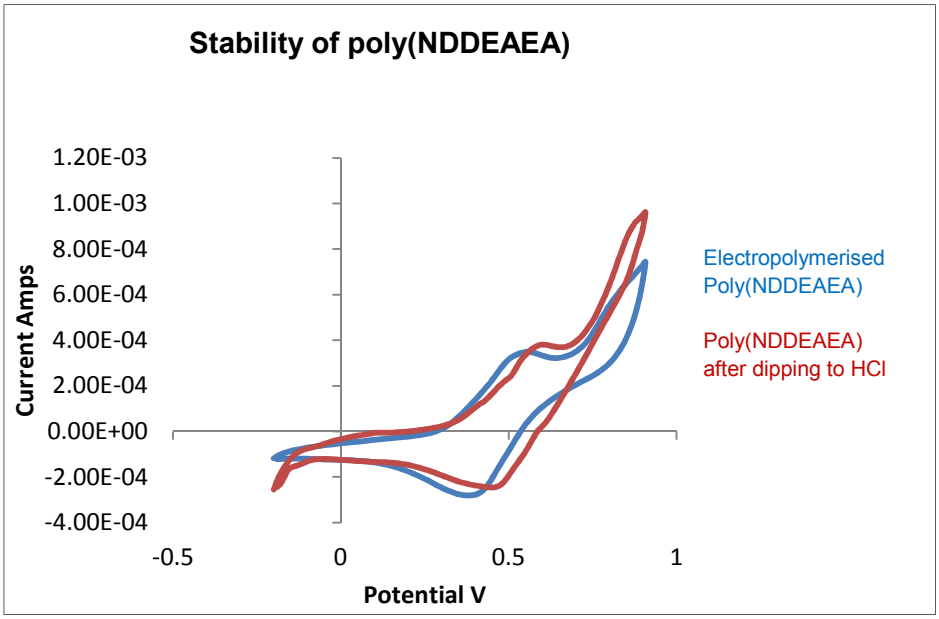


Figure 25 Stability of poly (NDDEAEA)

3.2.2 INIFETRER ACTIVATION AND PHOTOCROSSLINKING OF ELECTROPOLYMERISED FILMS OF NDDEAEA

When the Poly(NDDEAEA)-modified electrode introduced in 0.75M HCl and irradiated with UV light, a photochemical cleavage of the C-S bond occurred, and a pair of free radicals was formed. The free radicals present very different reactivities, including a reactive carbon-centered radical and a low reactivity (sometimes known as dormant) dithiocarbamate radical. This process is reversible. Upon UV irradiation, electropolymerised poly(NDDEAEA) generated dormant DTC radicals and active carbon centered radicals, thereby acting as an iniferter.

Figure 26 shows the cyclic voltammetric response of poly (NDDEAEA) before and after irradiation. The first voltammogram (A) observed is due to the cyclic voltammetric response of the electropolymerised poly (NDDEAEA). The second CV (B) is the cyclic voltammetric response of poly (NDDEAEA) after the first irradiation and the third one (C) is after the second irradiation. The oxidation peak currents obtained confirmed the formation of radicals due to UV light irradiation.

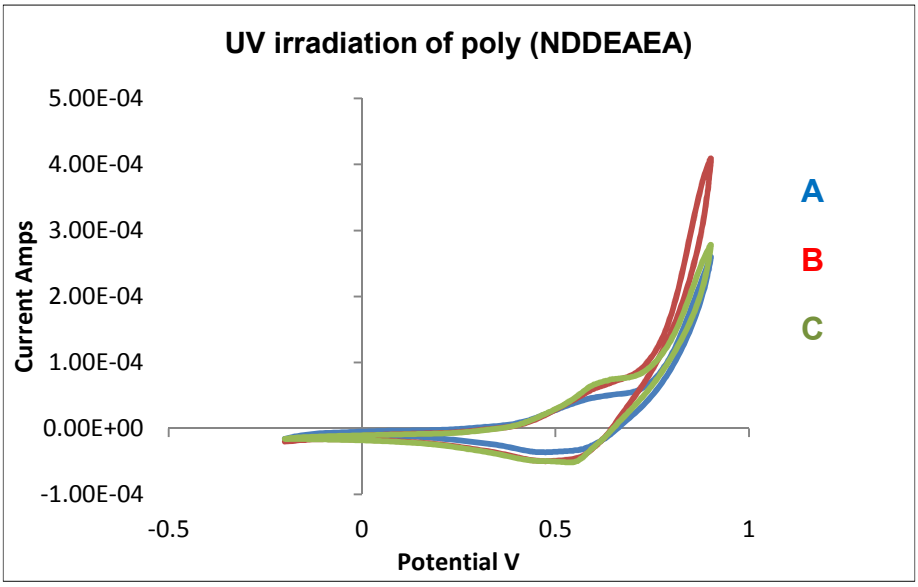


Figure 26 Cyclic voltammetric responses of poly (NDDEAEA) before and after UV irradiation

3.2.3 PHOTOGRAFTING OF VARIOUS MONOMERS ONTO ELECTROPOLYMERISED FILMS NDDEAEA

Surface macromolecular architectural designs using photografting experiments based on the photochemistry of DTC moieties of NDDEAEA were successfully demonstrated. Electropolymerised films of poly(NDDEAEA) in a monomer solution of

methacrylic acid, styrene or AMPSA in acetonitrile was UV irradiated under a nitrogen atmosphere for 20 minutes Photolysis of the iniferter component of poly(NDDEAEA) modified electrode surfaces by UV irradiation yields a pair of radicals, an active carbon centered radical which can react with another monomer such as MAA or styrene to yield a radical polymer end and a dormant DTC species which reacts weakly or not at all with these monomers but can undergo to termination reactions through recombination with a growing polymeric chain.(Figures 27 & 28)

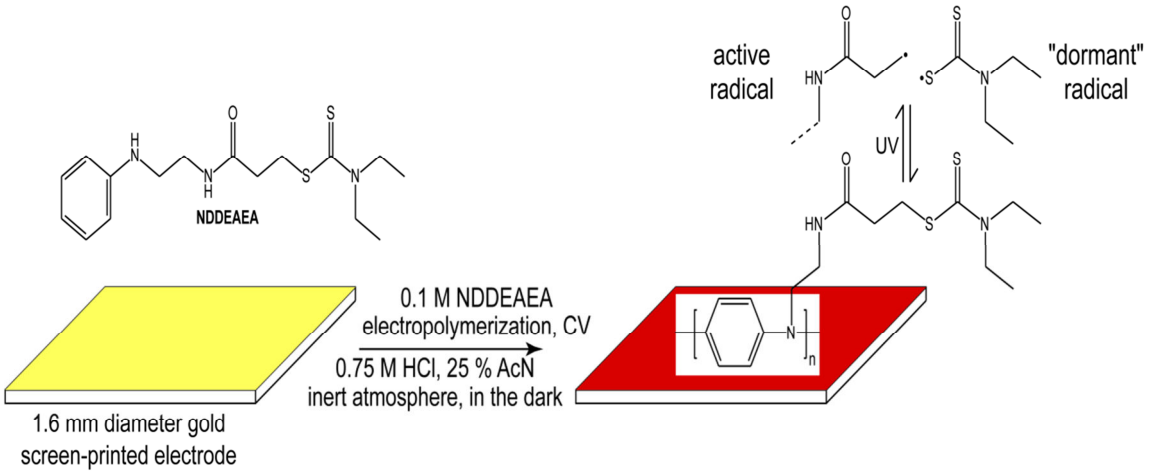


Figure 27 Electrochemical polymerisation of NDDEAEA giving rise to PANI (PANI) chains with pendant dithiocarbamate moieties.

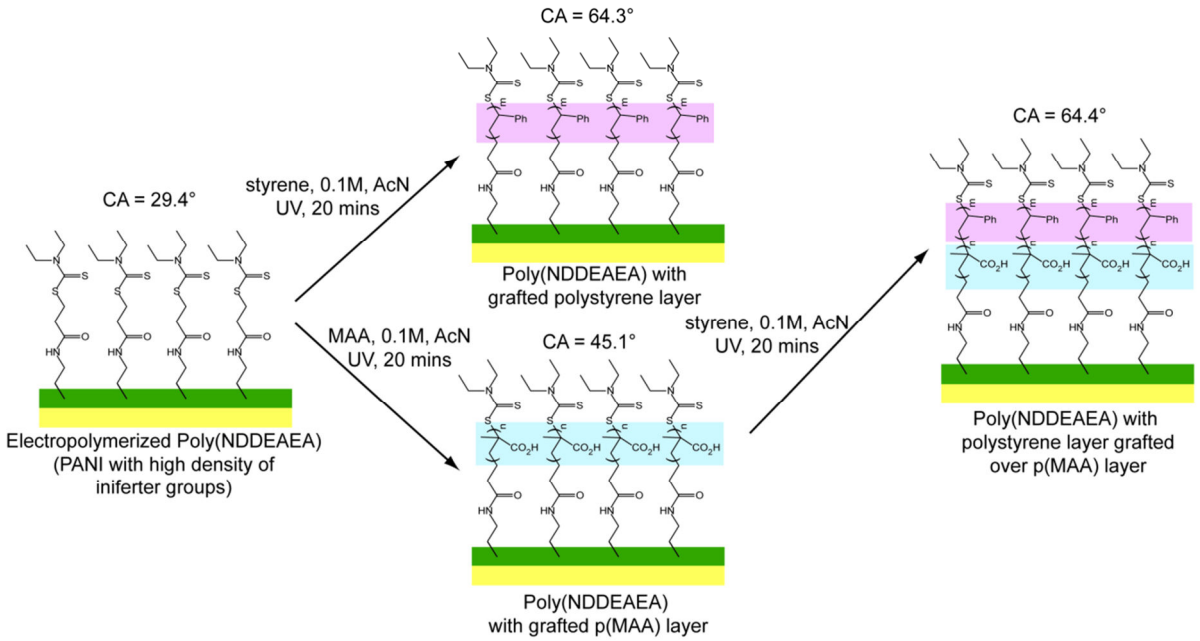


Figure 28 Scheme representative of photografting of various monomers onto electropolymerised film of NDDEAEA

Grafted polymeric surfaces were characterized by Contact Angle (CA) measurements. In Table 14, are presented the static contact angle measurements of various grafted polymeric surfaces.

Table 14 contact-angle measurements of various grafted polymeric surfaces

Nature of the surface	Water contact angle (º)
Pretreated Screen-printed gold Electrode (SPE)	66.06
Electropolymerised poly(NDDEAEA)	29.46
Electropolymerised poly(NDDEAEA after UV irradiation	23.75
Poly(MAA) surface-grafted to electropolymerised poly(NDDEAEA) film	45.12
Poly(AMPSA) surface-grafted to electropolymerised poly(NDDEAEA) film	33.07
Poly(Styrene) surface-grafted to electropolymerised Poly(NDDEAEA) film	64.4
Poly(Styrene) over poly(MAA) surface-grafted to electropolymerised poly(NDDEAEA) film (layer-by–layer grafting)	64.39

As expected, the contact angle measurements demonstrate that the electropolymerised poly (NDDEAEA) was quite hydrophilic with CA of 29.4º and became more hydrophilic after UV-irradiation (23.7º), this might be due to generation of free-radicals. Poly (MAA) grafted layer was moderately hydrophobic with a contact angle of 45.1º, while poly (AMPSA) was more hydrophilic (CA of 33.0 º) this might be due to its high acidic sulfonic acid moiety present in AMPSA. On the other hand, polystyrene layer was hydrophobic with contact angle of 64.4º. Thus, the contact angle measurements can be used to follow the growth of polymeric bilayer upon

reinitiation of the polymerization of a second monomer (Styrene) over the first one (MAA). The contact angle measurements (before and after the attachment of styrene) show quantitative changes in hydrophobicity as expected (CA of 64.5º).

The grafting of the monomers was also observed by SEM and AFM measurements. SEM (Figures 29-33) reveals a globular structure of the pre-treated SPE surface which displays an obvious change (some holes appear) associated with the electropolymerisation of NDDEAEA. After grafting of hydrophilic monomers, MAA and AMPSA over electropolymerised poly(NDDEAEA), the films show rather smooth surfaces and compact film structures compared to the surface with grafted styrene which displayed some uneven hollow structures. These results directly imply that the presence of acetonitrile enhances the phase separation and structure heterogeneity during the pore generation stage since styrene is a hydrophobic monomer.

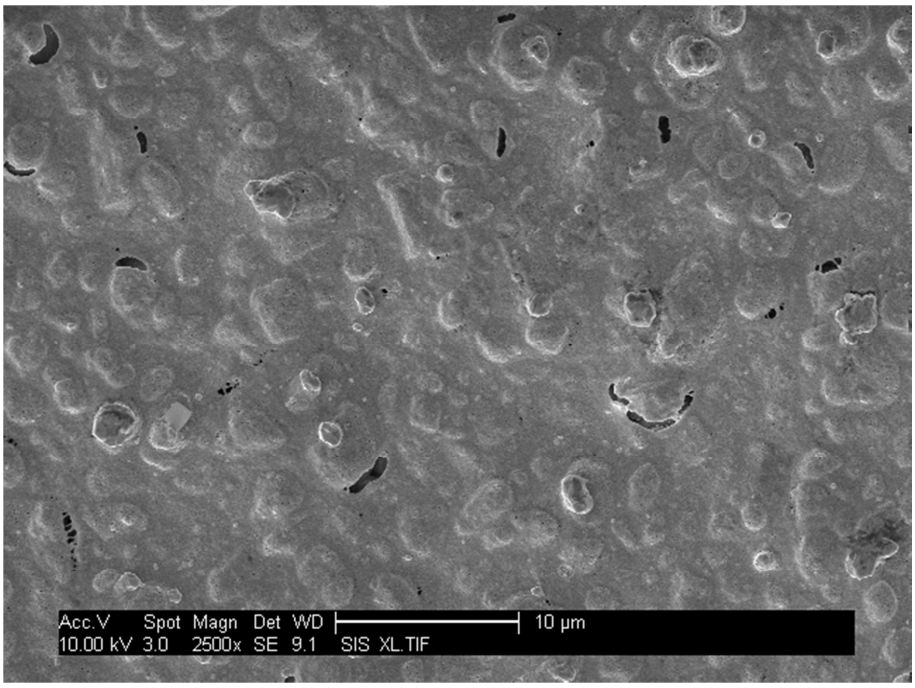


Figure 29 SEM image of surface morphology of pre-treated gold screen-printed electrode

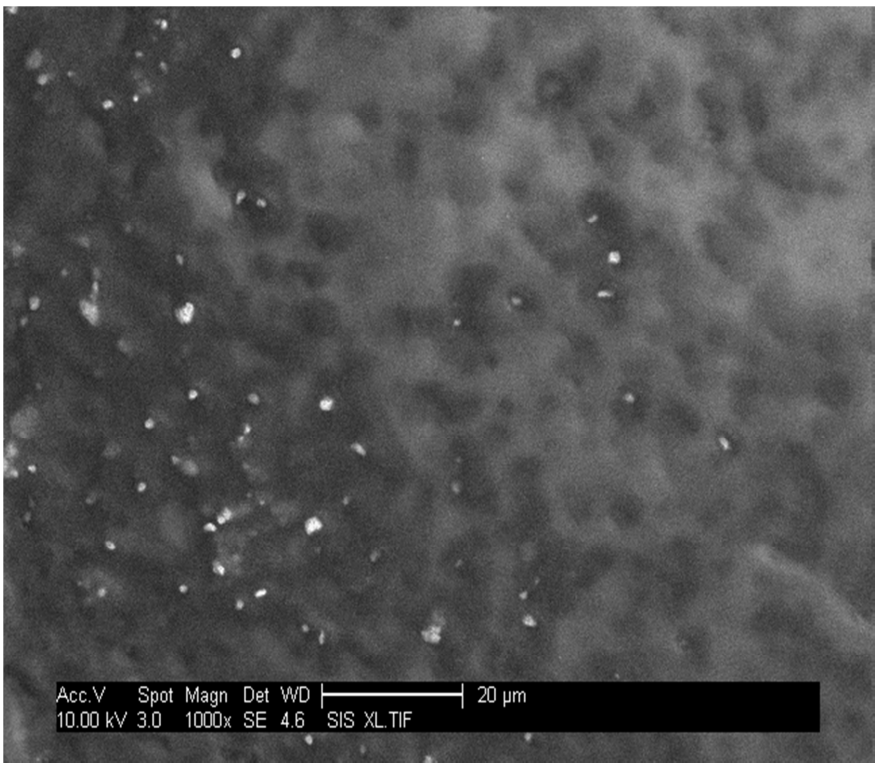


Figure 30 SEM image of surface morphology of electropolymerized NDDEAEA electrode

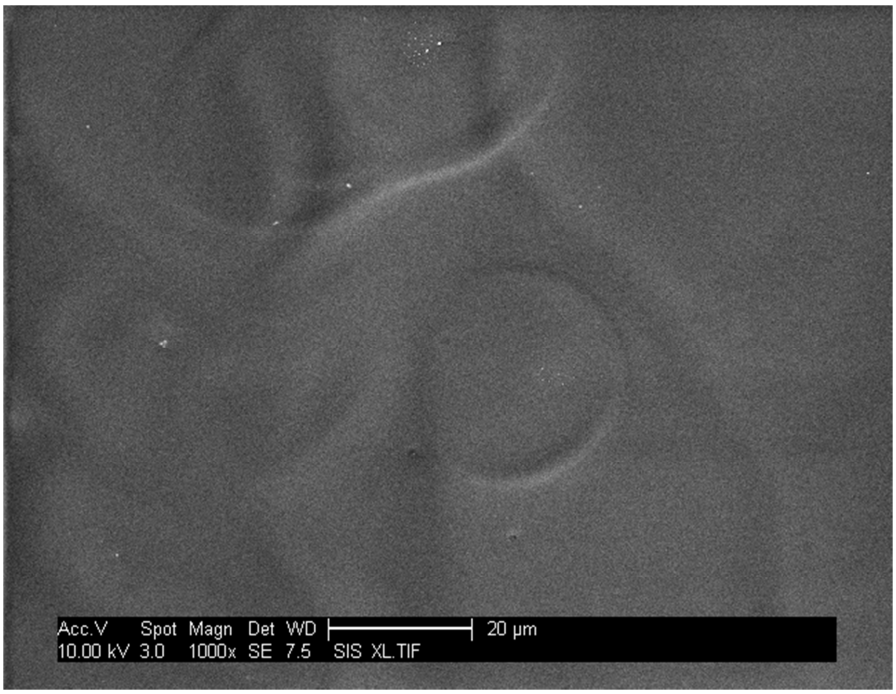


Figure 31. SEM image of surface morfology of electropolymerised NDDEAEA grafted with MAA

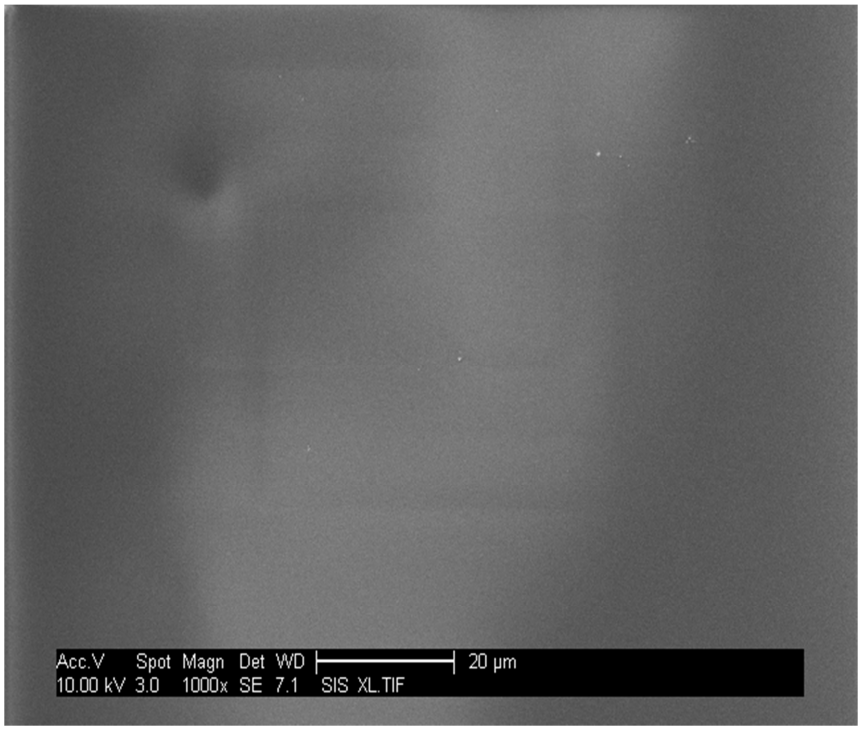


Figure 32 SEM image of surface morfology of electropolymerised NDDEAEA grafted with AMPSA

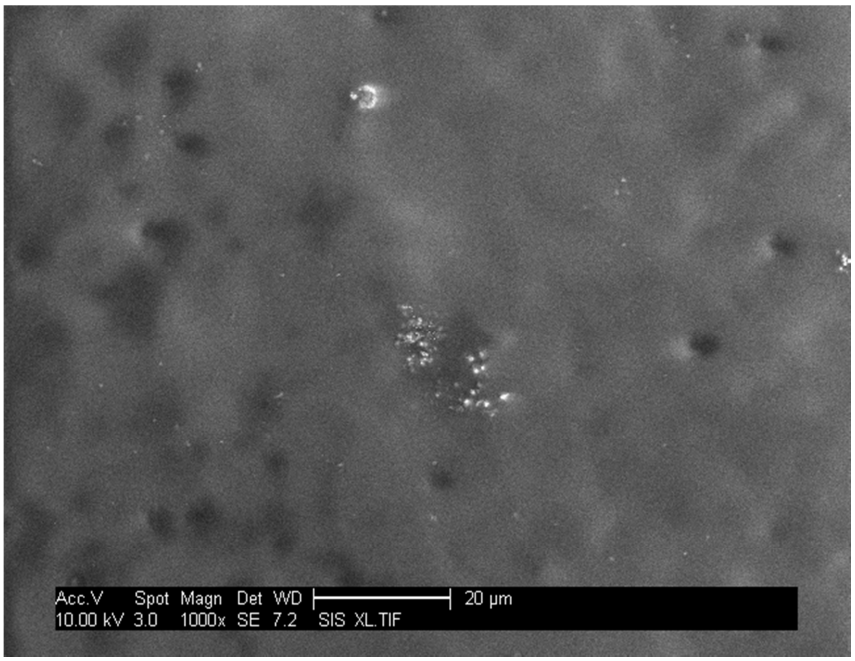


Figure 332 SEM image of surface morfology of electropolymerised NDDEAEA grafted with stryrene

AFM measurements are also made on various modified surfaces prepared by electropolymerisation of NDDEAEA and grafting of poly(MAA), poly(AMPSA) and polystyrene over an electropolymerised NDDEAEA layer (Figures 34-39). As can be seen, the bare gold electrode displays a typical grainy morphology over some hollow structure. Electropolymerisation of the NDDEAEA leads to deposition of polymer on the surface, smoothing out many of these features with some spikes on it which increases by UV irradiation. Following grafting with poly(methacrylic acid) and poly(AMPSA) by activating the dithiocarbamate part of the NDDEAEA, the surface became smoother. Meanwhile grafting of polystyrene over the electropolymerised NDDEAEA shows some holes and bumps in the polymer layer which might be due to phase separation in acetonitrile. Layer by layer grafting of polystyrene over

poly(methacrylic acid) also displayed this type of behavior which proves that the dithiocarbamate radical can be activated again due to UV irradiation.

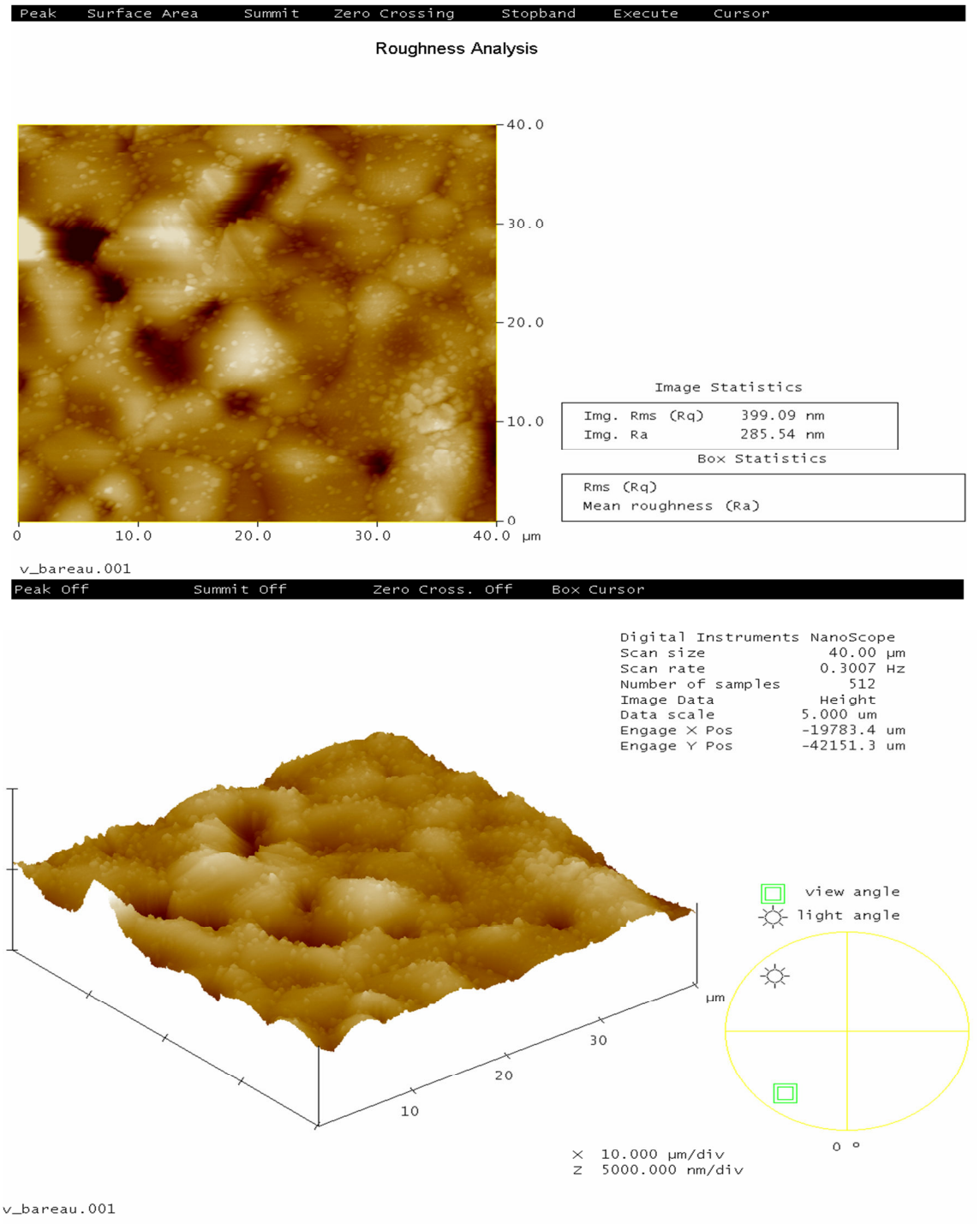


Figure 34 AFM of bare pre-treated gold SPE

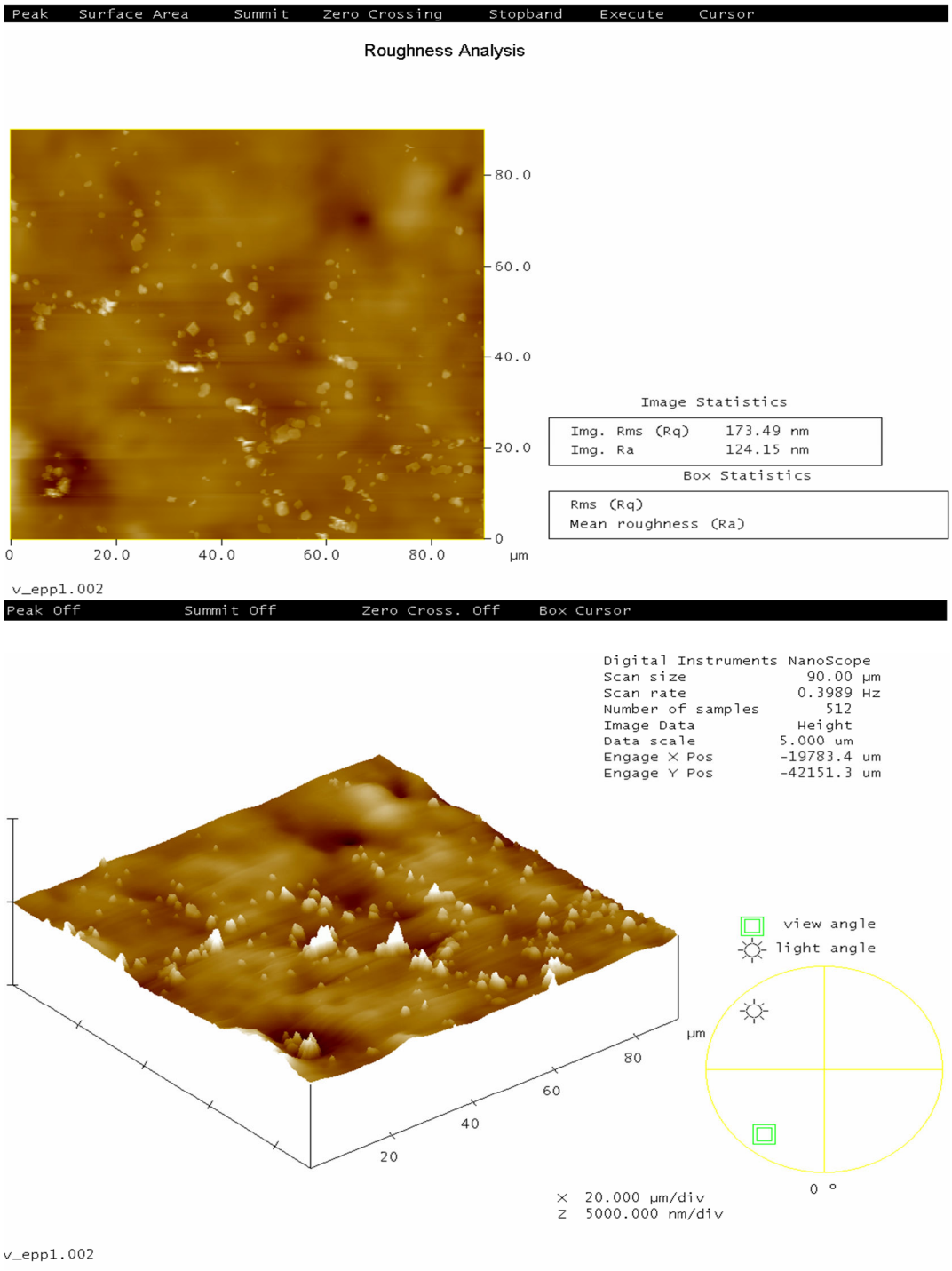


Figure 35 AFM of electropolymerised NDDEAEA film on pre-treated gold SPE after 15 min irradiation

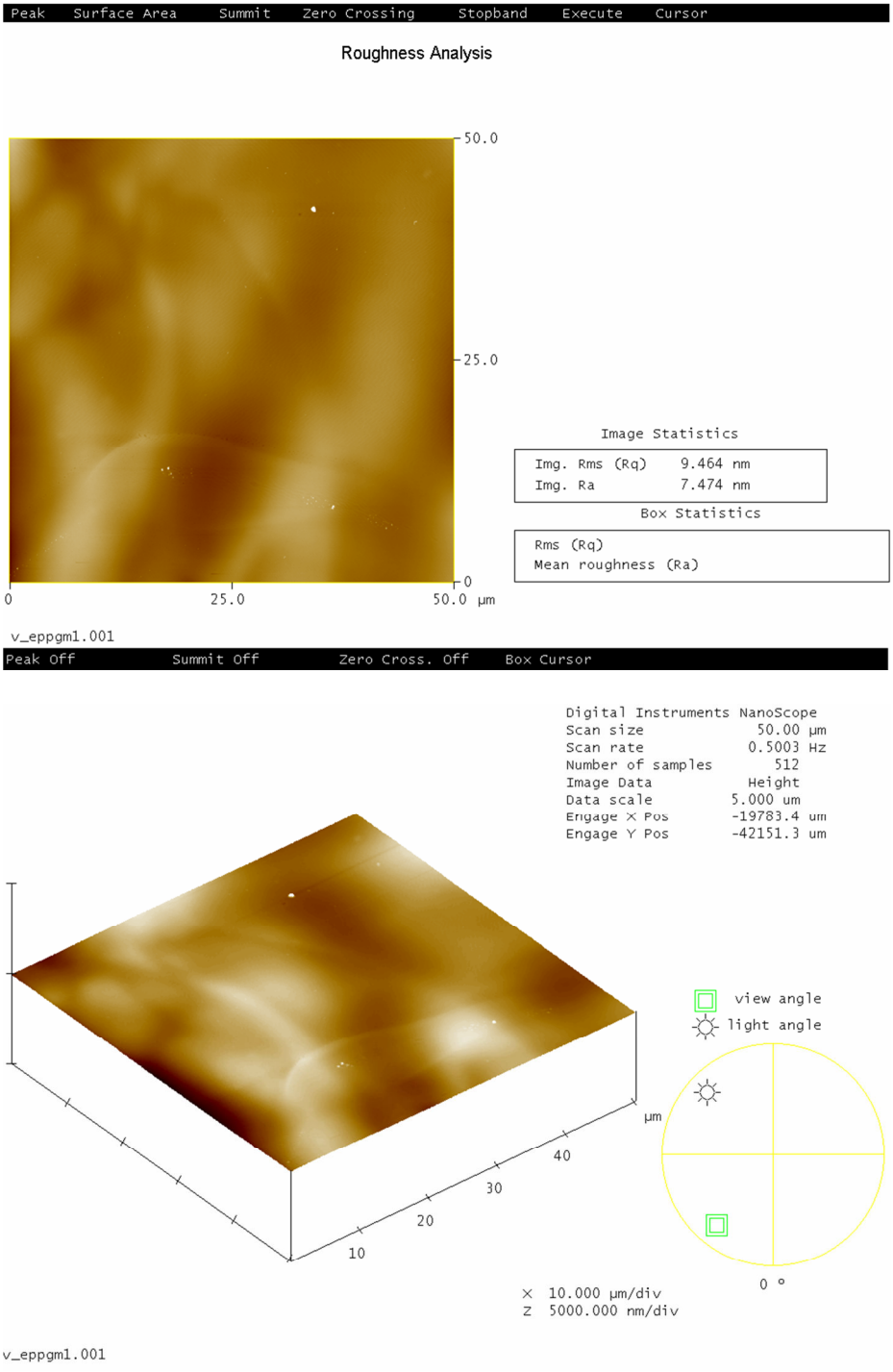


Figure 36 Poly (methacrylic acid) film grafted over electropolymerised NDDEAEA by iniferter activation using UV irradiation

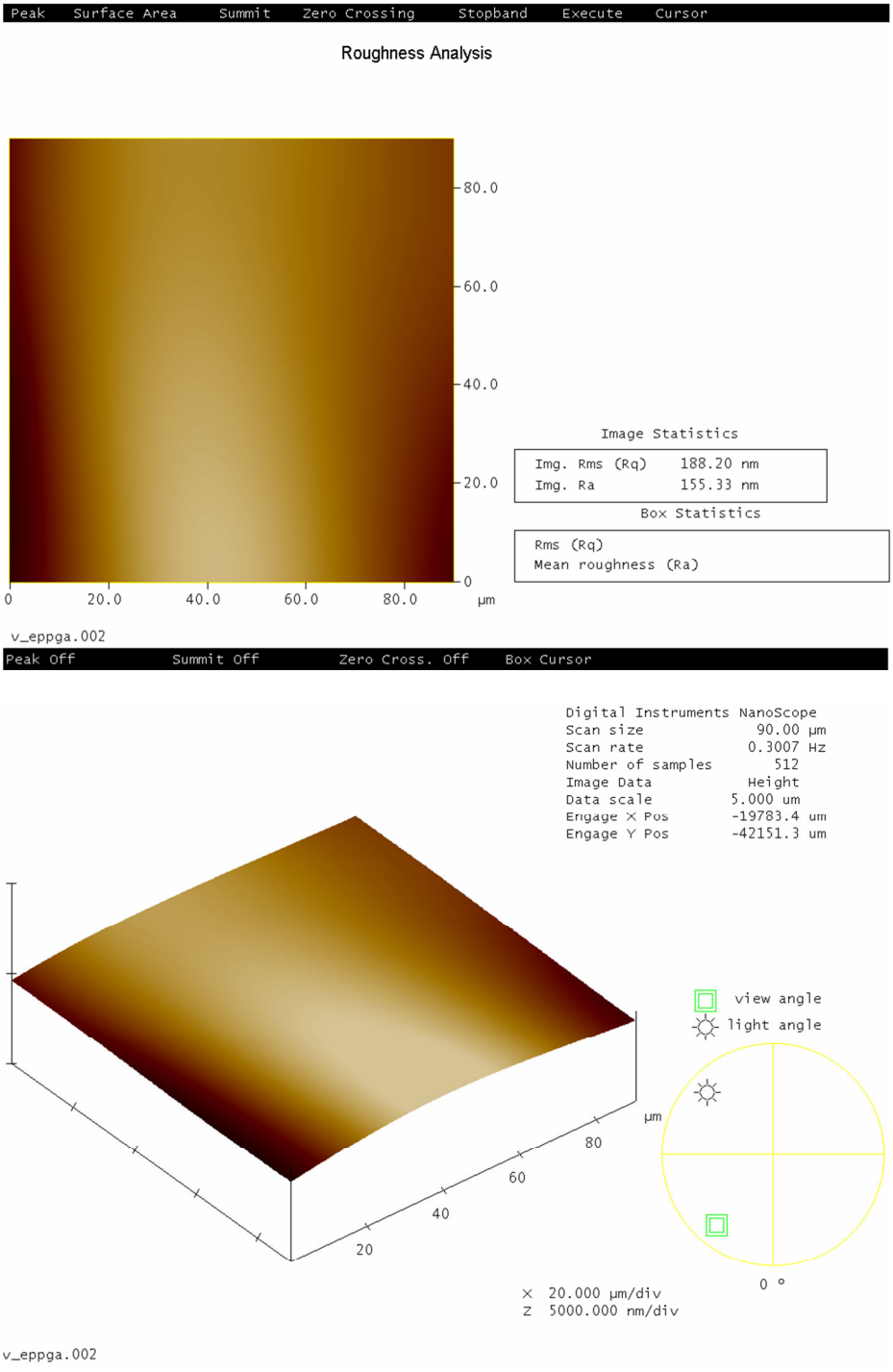


Figure 373 Poly (AMPSA) film grafted over electropolymerised NDDEAEA by iniferter activation using UV irradiation

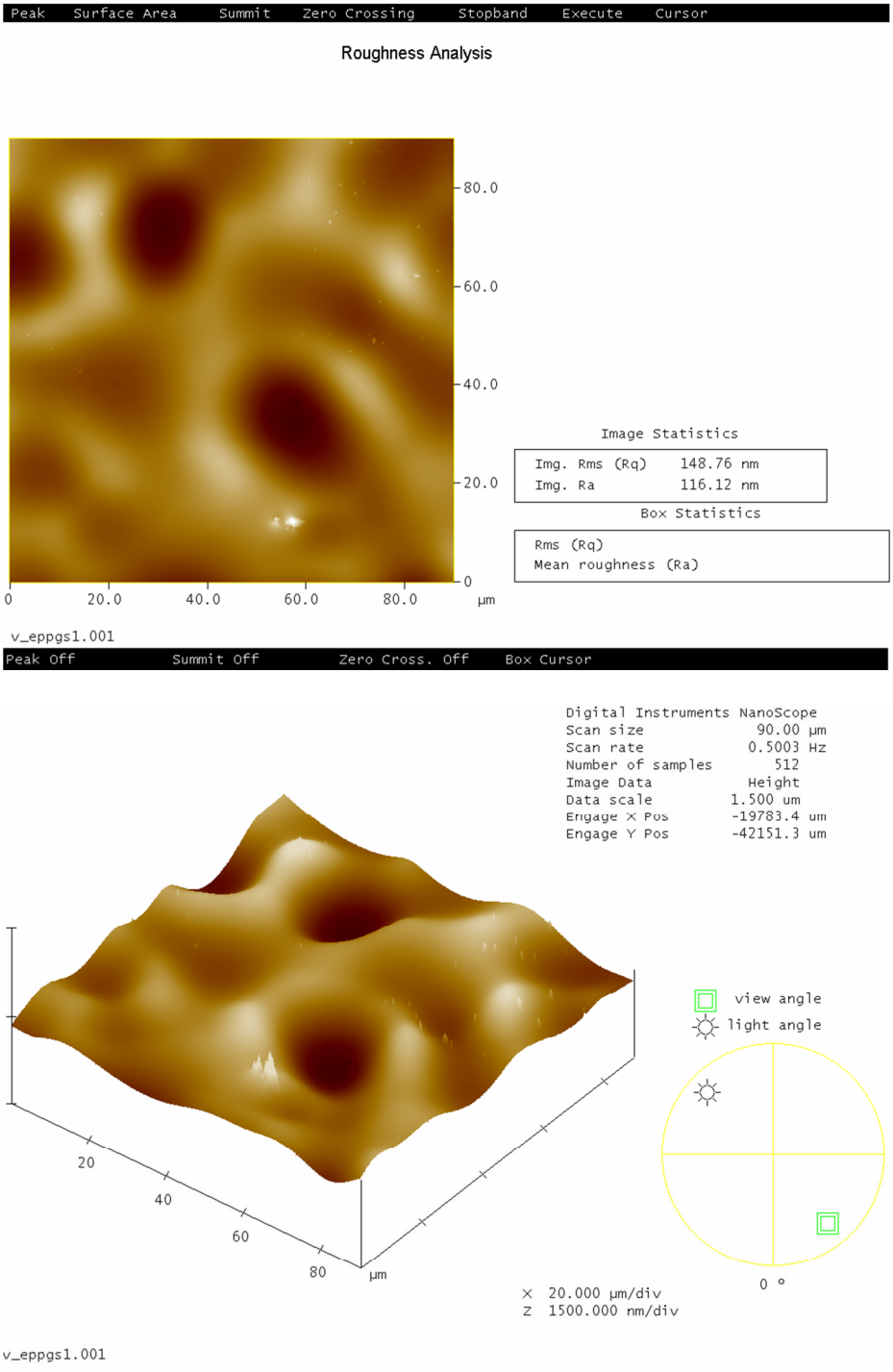


Figure 38 Poly (styrene) film grafted over electropolymerised NDDEAEA by iniferter activation using UV irradiation

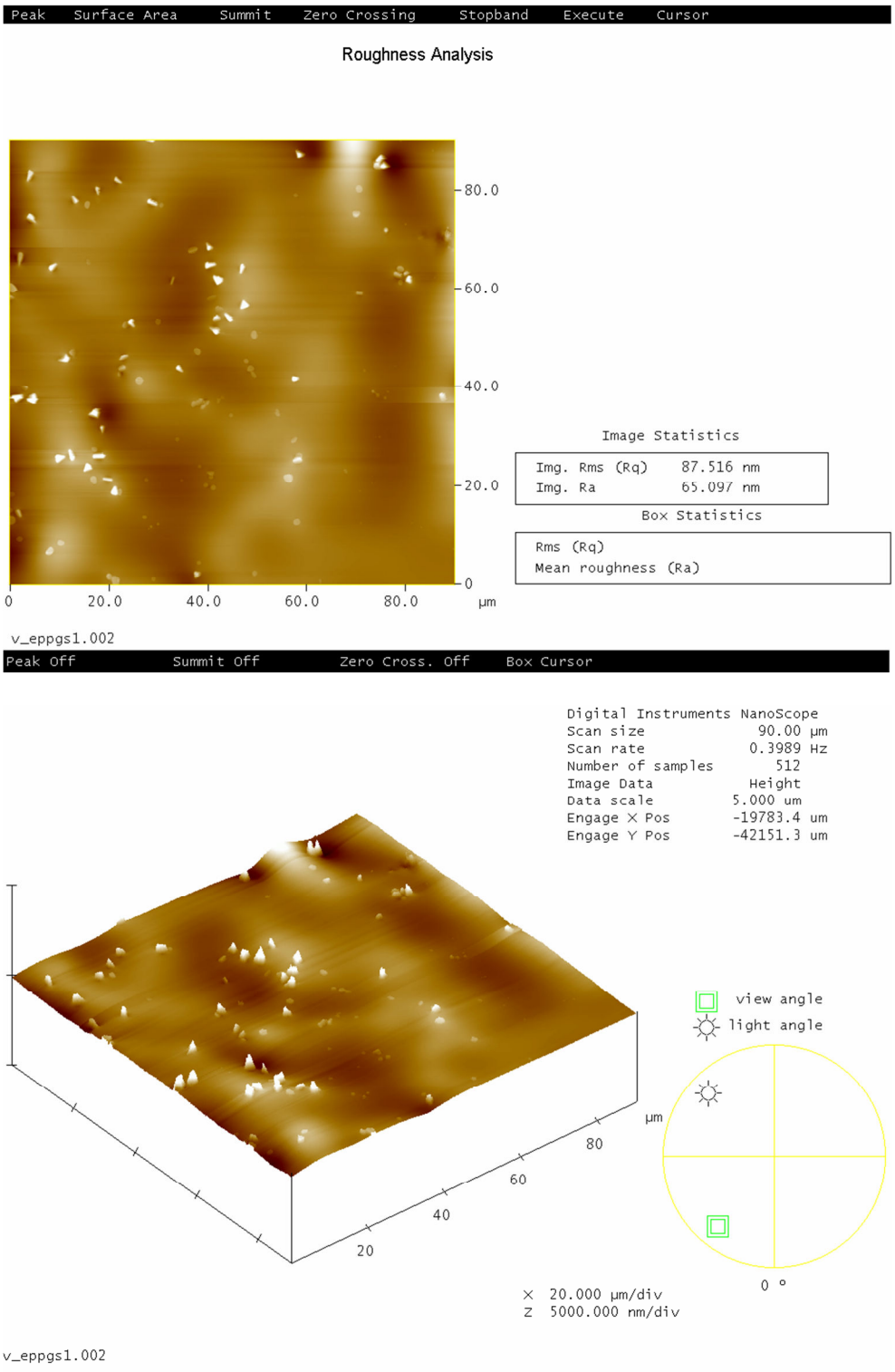


Figure 39 Layer by layer grafting of Poly (styrene) film over poly (methacrylic acid) over electropolymerised NDDEAEA by iniferter activation using UV irradiation

3.2.4 IMMOBILISATION OF GLUCOSE OXIDASE OVER ELECTROPOLYMERISED NDDEAEA BY INIFERTER ACTIVATION USING UV IRRADIATION

i) GOx loading

In order to immobilise GOx over electropolymerised NDDEAEA an electropolymerised poly (NDDEAEA)-modified SPE was placed horizontally in a glass vial and UV irradiated after applying 150 µL of different concentrations of GOx on the electrode surface, for 15 minutes. A maximum current was obtained when 30mg/ml GOx was used. The cyclic voltammetric response of immobilised GOx over electropolymerised NDDEAEA in the presence of glucose is presented in Figure 40.

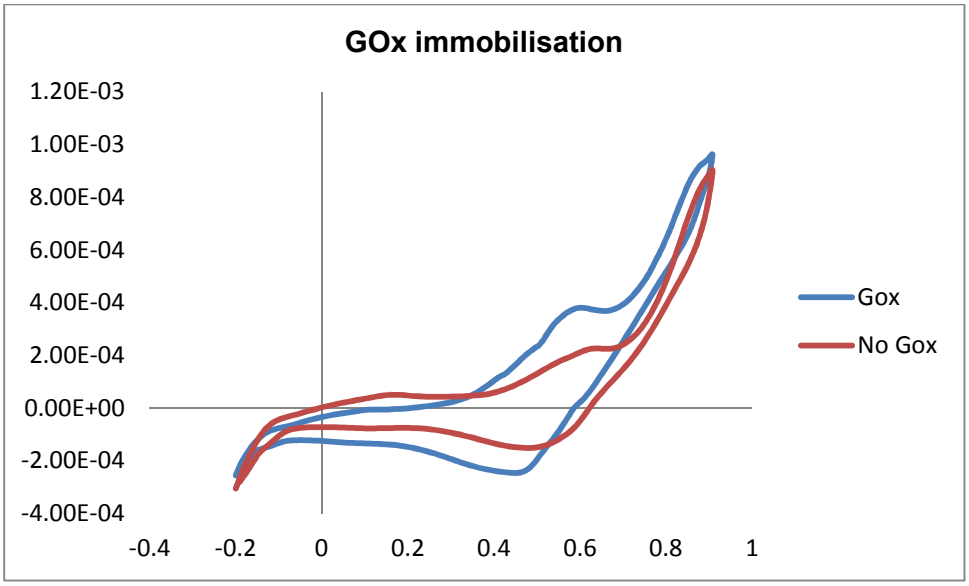


Figure 40 The cyclic voltammetric responses of immobilised GOx over electropolymerised NDDEAEA in the presence of glucose

The electrochemical response of the biosensor to glucose was examined using cyclic voltammetry. It was demonstrated that the current response of the NDDEAEA /GOx electrode measured in 0.1 M PBS pH 7.5 (the specific pH reflects the optimum conditions for enzymatic mediated electrochemical reactions) after adding different concentrations of glucose increases with the increased concentrations of glucose.

ii) Working Potential

The effect of applied potential on the amperometric response of the biosensor to glucose was investigated with the NDDEAEA /GOx electrode to glucose. In Figure 41 is presented the dependence of the amperometric response current to 0.5mM injection of glucose. The applied potential tested varied from 0 to 0.6V. The response increased when the applied potential was increased from 0 to 0.4V, while further increase of the applied potential, resulted in the decrease of the amperometric response. In order to minimize interferences and achieve the lowest detection limit the working potential was set at 0.35V, as the optimum applied potential for the amperometric measurements.

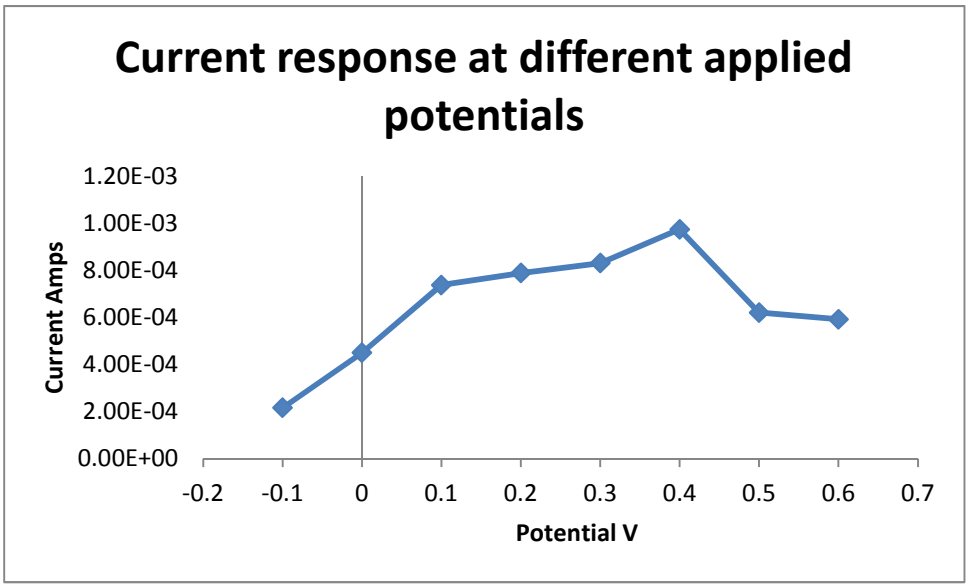


Figure 41 Current response at different applied potentials

iii) Glucose calibration curve

Figure 42 shows the calibration curve of NDDEAEA /GOx electrode obtained in 0.1M PBS pH 7.5 at a potential of 0.35V with the consecutive additions of glucose. The specific biosensor presents a linear response to glucose concentration within the range from 0.1mM to 5 mM with a correlation coefficient of 0.9775. When glucose concentration is higher than 20mM plateau current was observed. The sensitivity of the biosensor is 3.293μA mM^{-1}, while the limit of detection is 69.81μM.

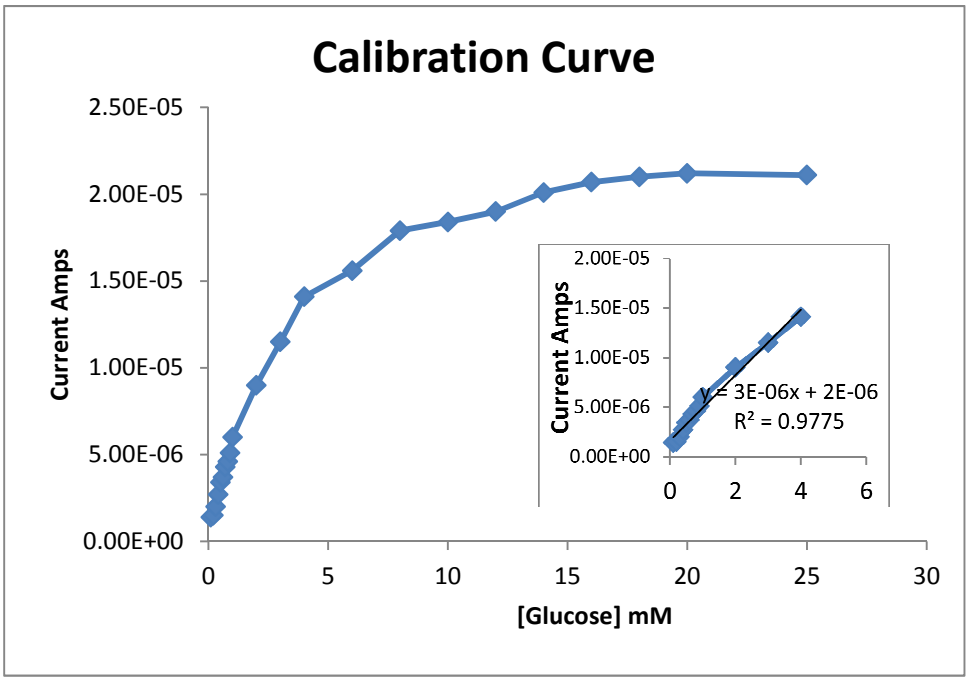


Figure 42 Calibration curve of NDDEAEA /GOx electrode

iv) Interference study

Some substances existed in biological samples could interfere with the detection of glucose. Ascorbic acid, sucrose and uric acid are the most common reducing agents present in biological samples, which may be electrochemically oxidized and interfere with the detection of glucose.

Figure 43 shows the current response of NDDEAEA /GOx electrode for the addition of 0.5mM of glucose in 0.1M PBS pH 7.5, followed by consecutive additions of ascorbic acid, sucrose and uric acid. (concentration range 0.1mM to 10mM)

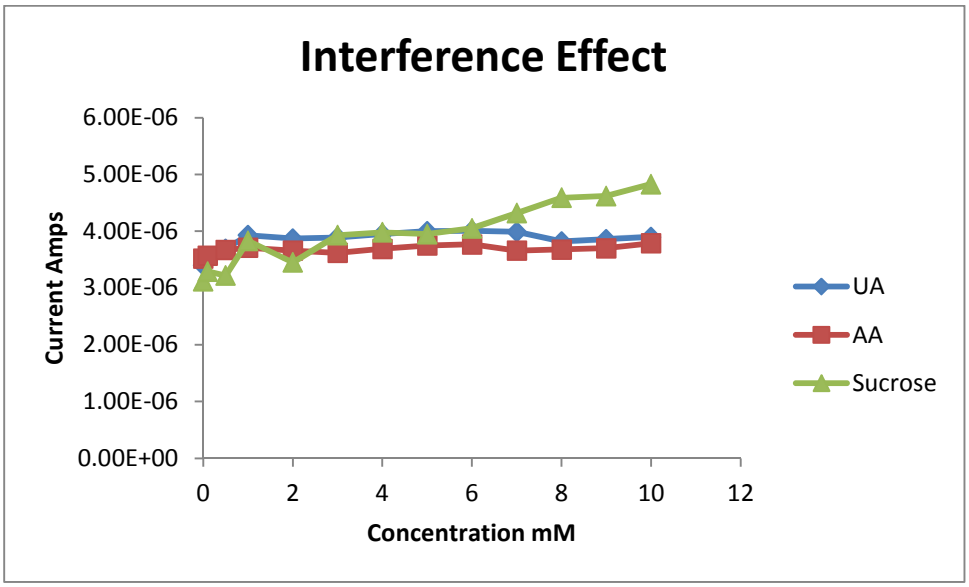


Figure 43 Current response of NDDEAEA /GOx electrode for the addition of 0.5mM of glucose, followed by consecutive additions of ascorbic acid, sucrose and uric acid. (concentration range 0.1mM to 10mM)

Table 15 Interference of uric acid, ascorbic acid and sucrose on response to glucose at NDDEAEA /GOx electrode at 0.35V working potential.

Ratio of Glucose to interferents	Uric Acid	Ascorbic Acid	Sucrose
1:1	8.53%	4.26%	3.21%
1:2	15.60%	5.40%	22.80%
1:10	17.65%	6.53%	26.60%
1:20	14.71%	7.67%	54.81%

It is observed that uric acid, ascorbic acid and sucrose in one to one ratio with glucose affected the amperometric response to a small extend, up 8.5% for uric acid, 4.26% for ascorbic acid and 3.21% for sucrose. It is worthy to note though that the

usual concentration of uric acid in the blood sample is 0.116 mM to 0.472mM, while for ascorbic acid is 0.022mM to 0.083mM. Consequently the specific interference compounds will not affect the sensor response.

3.3 DEVELOPMENT AND OPTIMISATION OF THE REACTION CONDITIONS TO GRAFTING HYPERBRANCHED POLYMER ONTO THE SURFACE OF THE MULTI-WALLED CARBON NANOTUBES

The purpose of this study was to develop and optimise the reaction conditions to grafting a hyperbranched polymer onto the surface of the multi-walled carbon nanotubes (MWCNT), using the $A_3 + B_2$ approach. By this study we aimed to explore a further advanced approach to significantly increase the sensitivity of Pelikan SPEs.

3.3.1 GRAFTING AND CHARACTERISATION OF HYPERBRANCHED POLYMER ONTO MWCNTs

Initially the pristine MWCNT were acidic treated in order to produce carboxylic acid functionalised MWCNTs. The MWCNT-COOH was suspended in $SOCl_2$ in order to generate MWCNT-COCl. And finally the aminated MWCNTs were produced by reacting MWCNT-COCl with $H_2NC_2H_4NH_2$. The peaks retrieved in the IR spectra shown in Figure 44 are in agreement with the desired results. In more details the carbonyl bond (C=O) assigned to oxidised MWCNTs (MWNT-COOHs) occurs at 1720 cm^{-1}, while the absorption at 3430 cm^{-1} indicates the presence of hydroxyl groups. Moreover the N-H stretching absorption is less sensitive to hydrogen bonding than are O-H absorptions. The peaks retrieved at 1000 to 1350 cm-1 are

attributed to the C-N stretching absorptions. The results of FTIR spectra confirm the desired functionalisation/modification of the raw MWNT after treatment.

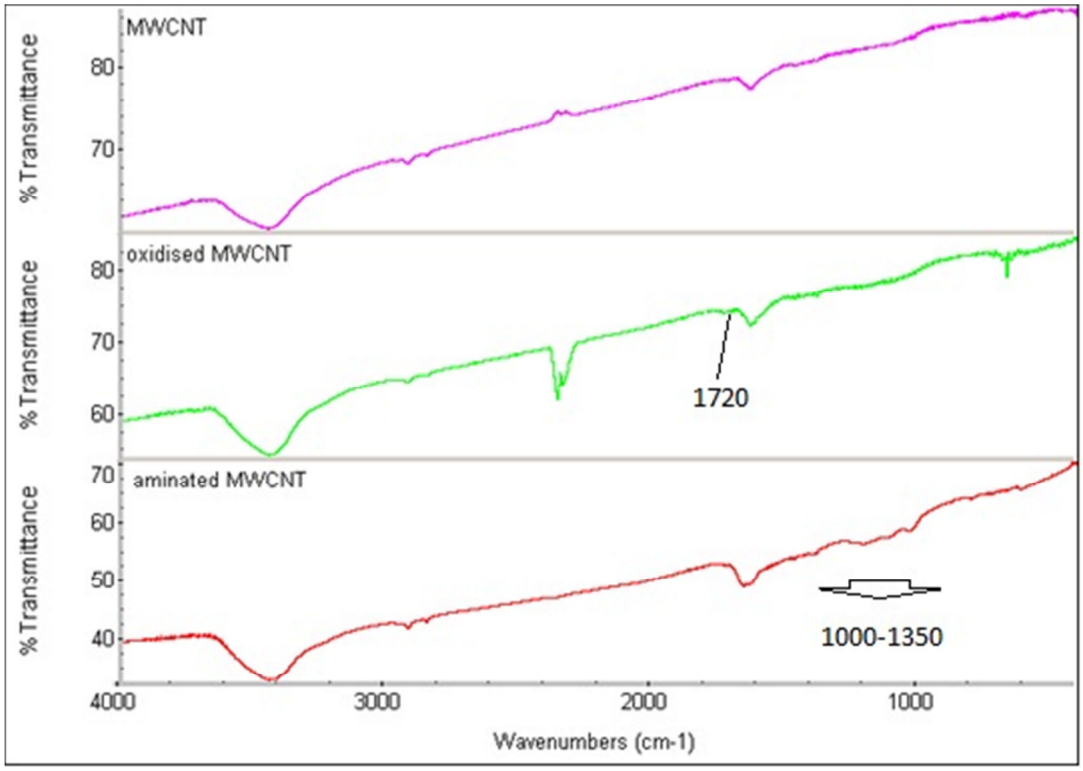


Figure 44 FT-IR spectra of MWCNTs

The SEM images of pristine (A), oxidised (B) and aminated (C) MWCNTs are shown in Figure 45. As can been seen the pristine MWCNTs present smooth surfaces in comparison with the treated MWCNTs.

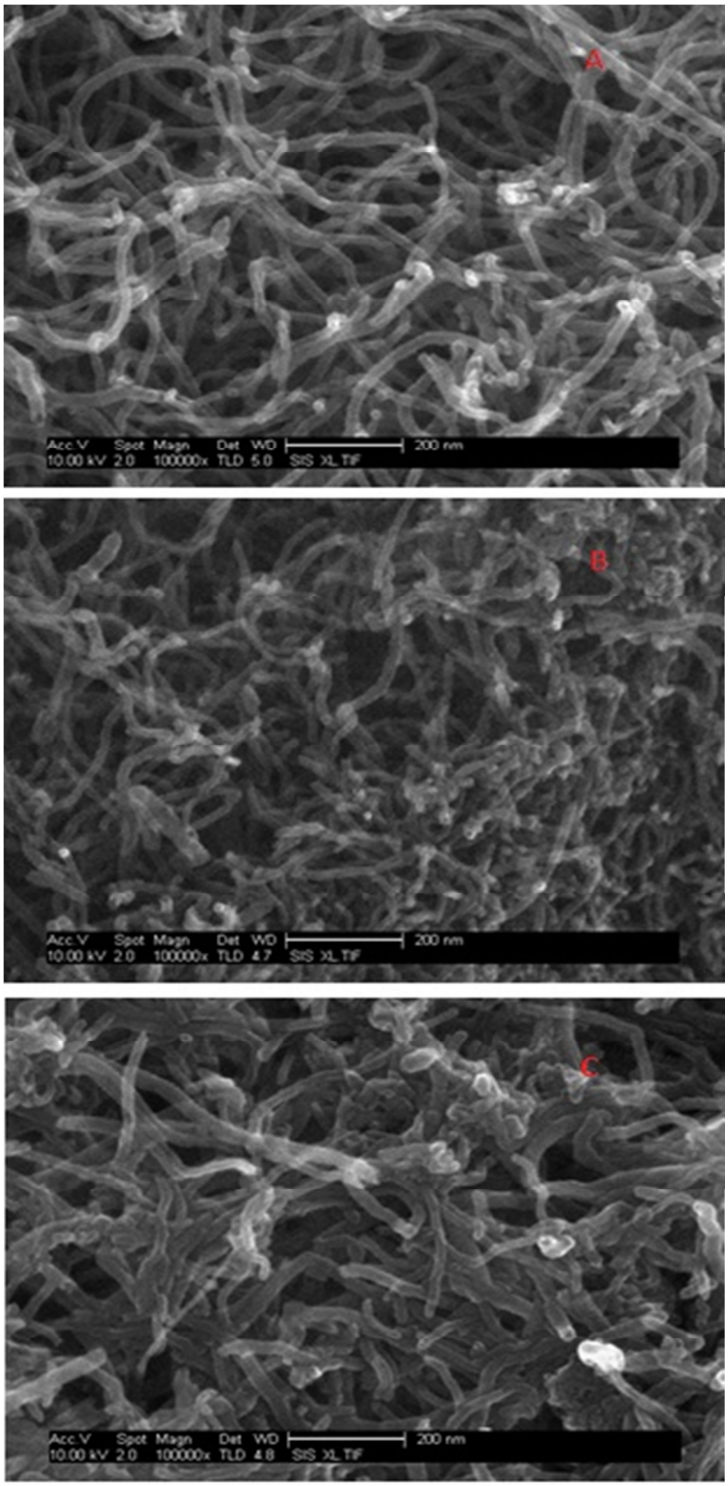


Figure 45 SEM images of pristine (A), oxidised (B) and aminated (C) MWCNTs. (magnification: 100kx and scale bar:200nm)

The molecular architecture of three dimensional macromolecules such as the hyperbranched polymers present a globular format. (***79***) Hyperbranched polymer attached to the MWCNTs can be observed in the SEM images that follow.

i) First approach of grafting a hyperbranched polymer onto MWCNTs using the A_3+B_2 approach

On the first approach the aminated MWCNTs (10wt%) were introduced in the ultrasonic bath for 15 minutes in the presence of 100 mL of dried ethanol. The A_3 (1,1′-Ferrocenedicarboxaldehyde) and B_2 (Tris(2-aminoethyl)amine) monomers (molar ratio 1:1) were added and stirred at room temperature for 30 minutes. After they were refluxed for 8 or 24 hours at 80 ^{o}C, they were let to cool down at room temperature. The reduction step was then followed by adding a 2.5eq. of sodium borohydride. Finally the mixture was submitted to vigorous stirring for 24 hours. The product was collected by suction filtration, using a 0.22μm polycarbonate membrane and further Soxhlet extracted in tetrahydrofuran for 24 hours at 66 ^{o}C. The final product was dried in the vacuum oven at 40^{o}C for 24 hours.

Figure 46 shows that the hyperbranched polymer grafted onto MWCNTs using the A_3+B_2 approach by setting the reflux time at 8 hours, while Figure 47 shows the hyperbranched polymer grafted onto MWCNTs using the A_3+B_2 approach by setting the reflux time at 24 hours. It was observed that many carbon tubes were aggregated to form bundles. Due to the polar surfaces of the MWCNTs, attributed to the amino groups present, there is an assembling tendency to form tetragonal or hexagonal packing. The average diameter of the modified MWCNTs is approximately 100nm, which is approximately three times thicker than the

unmodified MWCNTs.Thus it is suggested that by modifying the reflux time of the reaction, an overall increased degree of aggregation has been noticed.

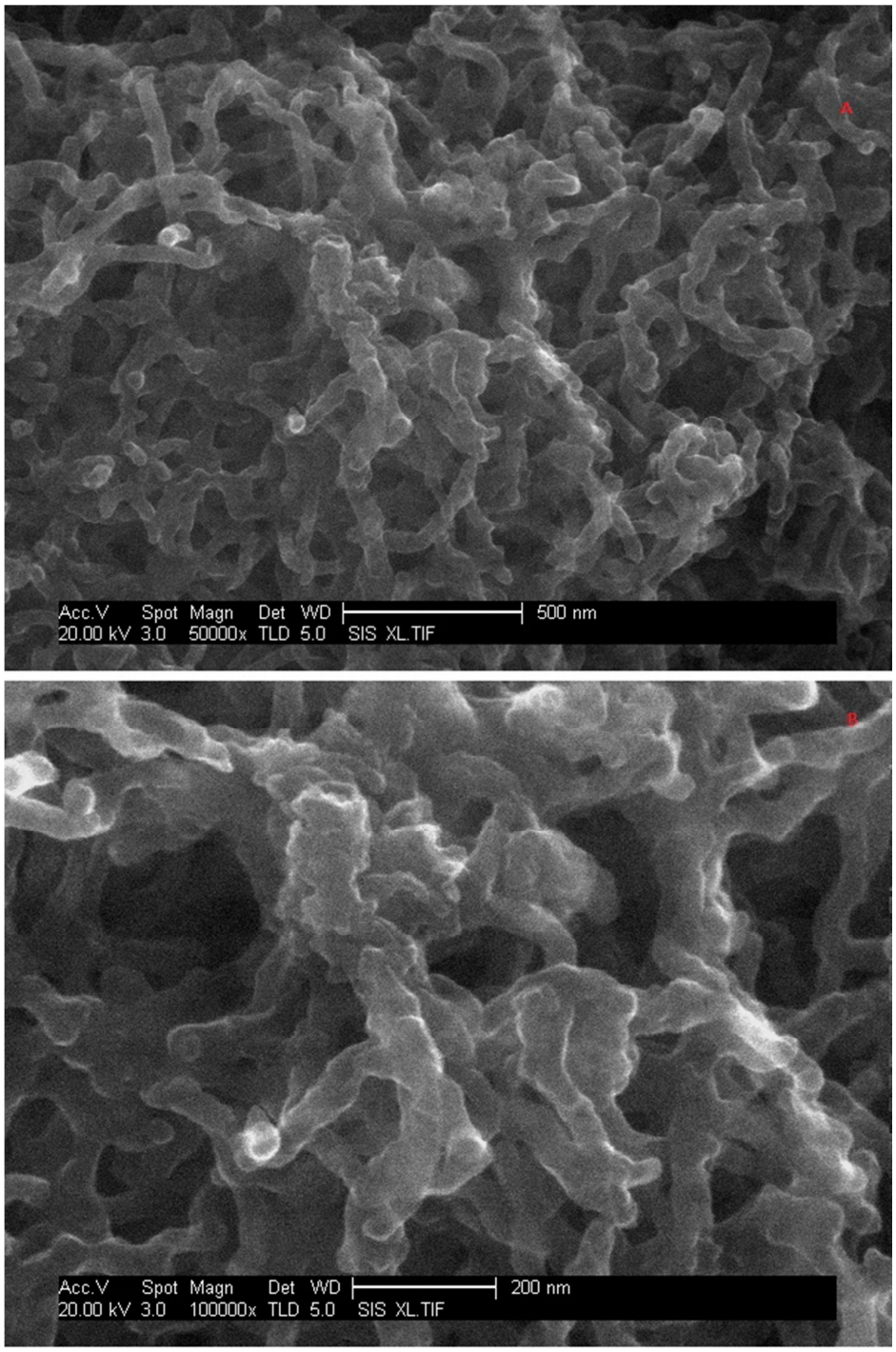


Figure 46 Hyperbranched polymer grafted onto MWCNTs using the A_3+B_2 approach (1^{st} method-reflux time for 8 hours)

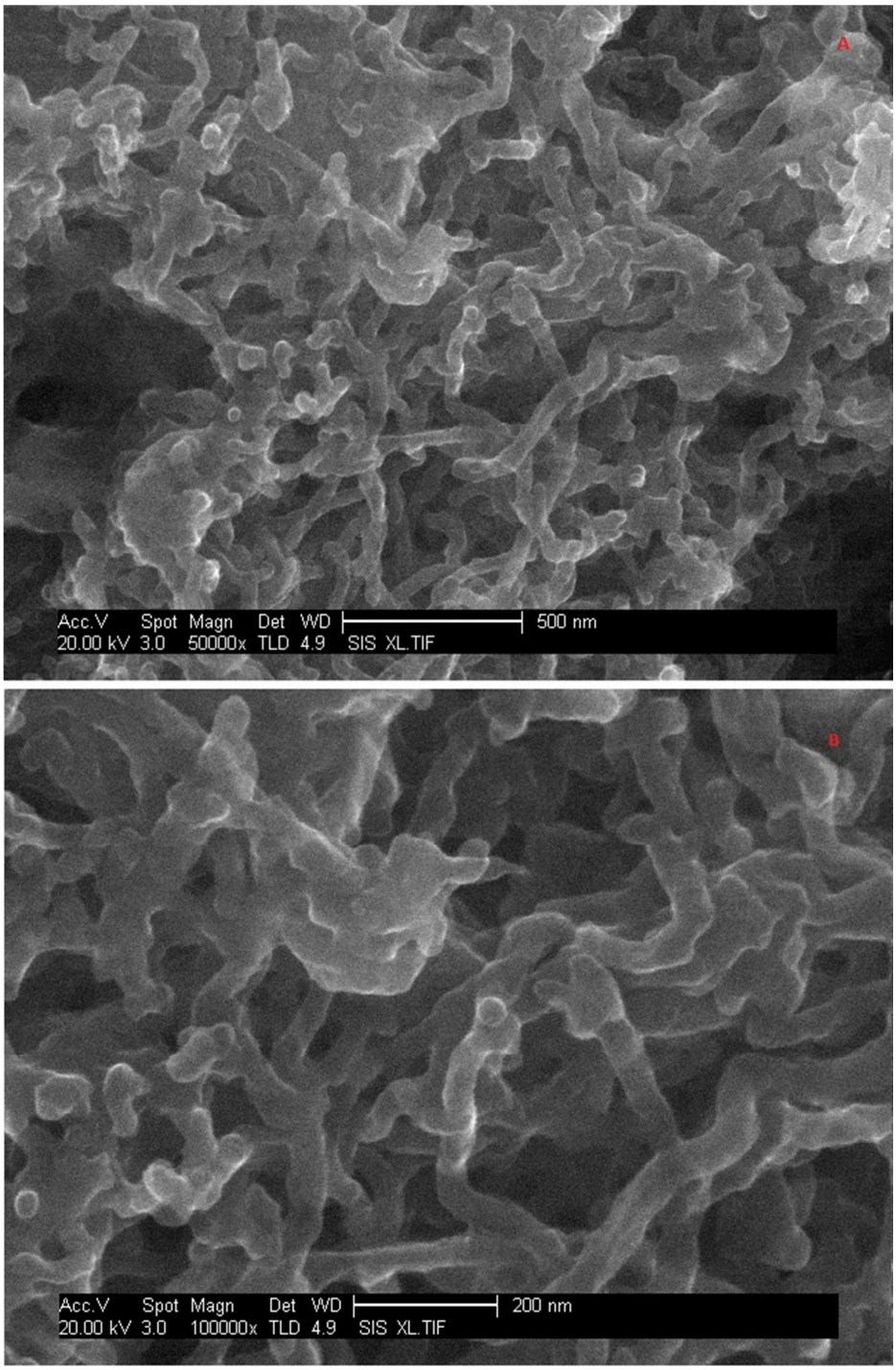


Figure 47 Hyperbranched polymer grafted onto MWCNTs using the A_3+B_2 approach (2^{nd} method-reflux time for 24 hours)

ii) Second approach of grafting a hyperbranched polymer onto MWCNTs from A_3+B_2 approach

The aminated MWCNTs (10wt%) were introduced in the ultrasonic bath for 15 minutes in the presence of 100mL of N-Methyl-2-pyrrolidon. 30mM of B_2 (Tris(2-aminoethyl)amine) monomer was mixed with two catalysts: the triphenylphosphit and the pyridine in a concentration 10 times higher than the concentration of the monomer and refluxed at 90 ^{o}C for 1 hour. After the addition of 30mM of A_3 (1,1′-Ferrocenedicarboxaldehyde) monomer and calcium chloride, in a 3 times higher concentration of the starting material, the mixture was allowed to reflux at 90 ^{o}C for 8 or 24 hours. The product was collected by suction filtration, using a 0.22μm polycarbonate membrane and further Soxhlet extracted in tetrahydrofuran for 24 hours at 66 ^{o}C. The final product was dried in the vacuum oven at 40^{o}C for 24 hours

Figure 48 shows the hyperbranched polymer grafted onto MWCNTs using the A_3+B_2 approach by setting the reflux time at 8 hours, while Figure 49 shows the hyperbranched polymer grafted onto MWCNTs using the A_3+B_2 approach by setting the reflux time at 24 hours. It was observed that many tubes were aggregated to form bundles, but no formations of polymers are visible on the surfaces of the MWCNTs.

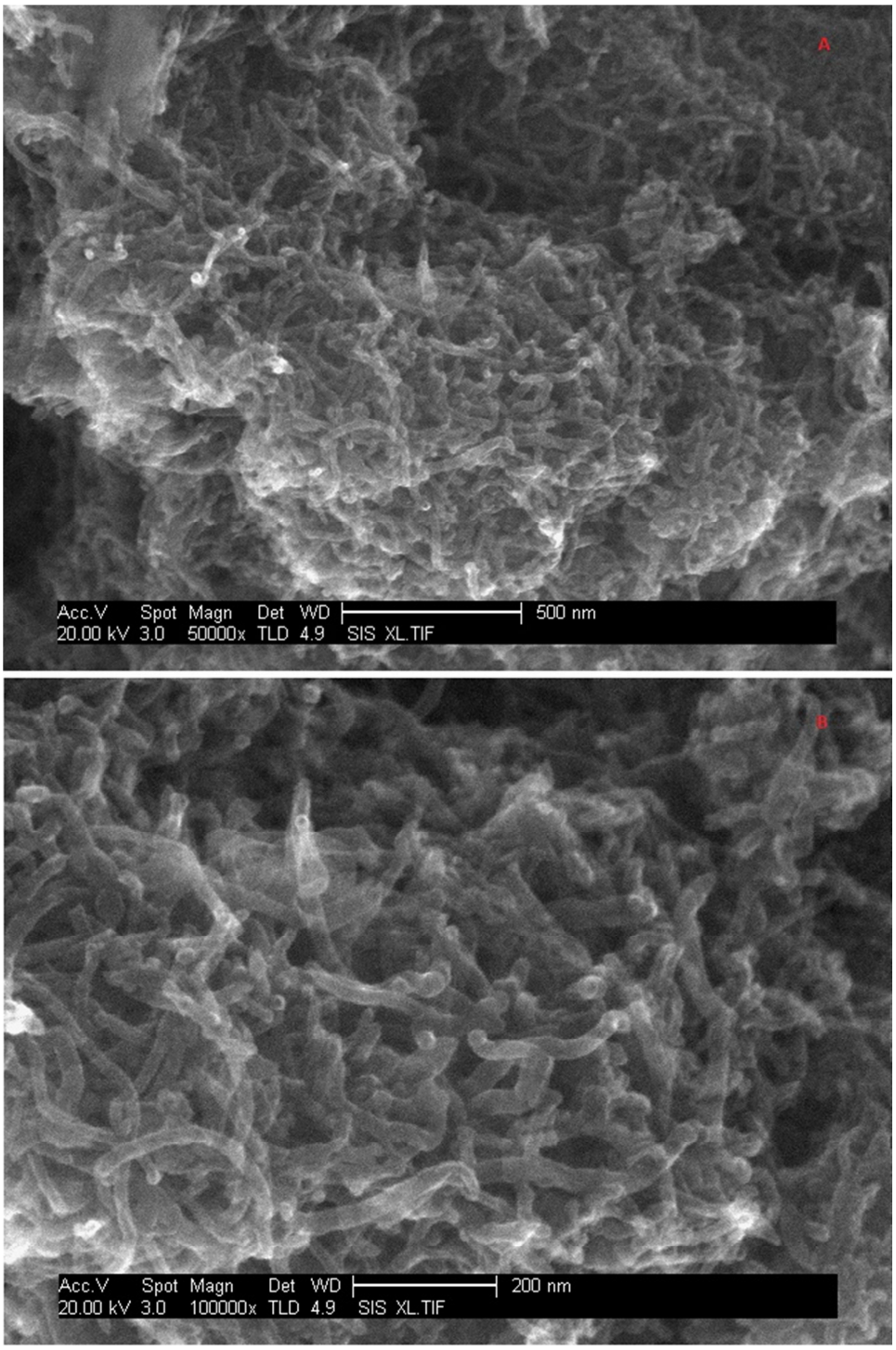


Figure 48 Hyperbranched polymer grafted onto MWCNTs from A_3+B_2 approach (2nd method-reflux time at 8 hours)

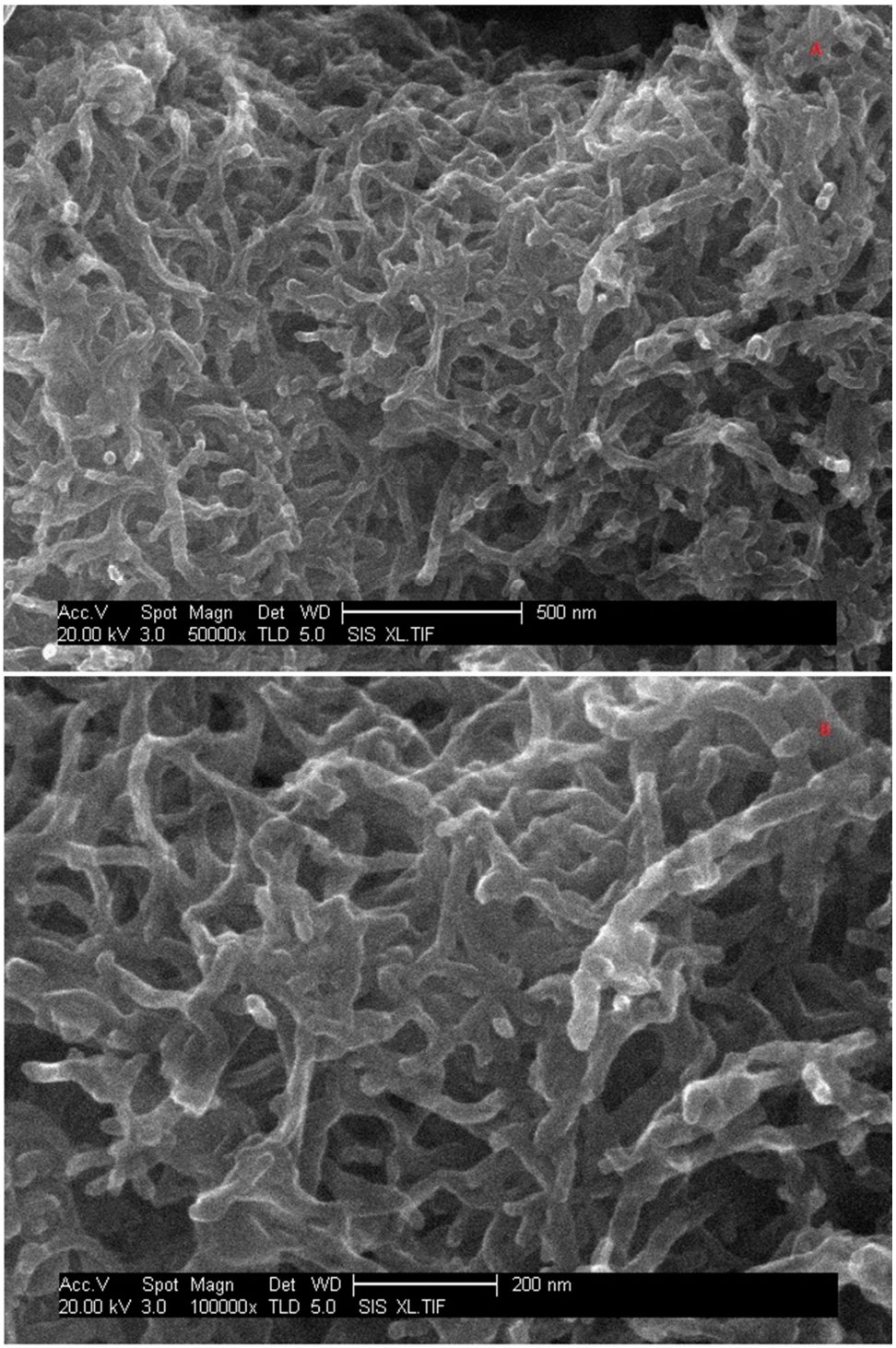


Figure 49 Hyperbranched polymer grafted onto MWCNTs from A_3+B_2 approach (second method-reflux time at 24 hours)

4TH CHAPTER

DISCUSSION

4.1 PELIKAN SUN: A COMMERCIAL AMPEROMETRIC GLUCOSE OXIDASE BIOSENSOR

4.1.1 DETERMINATION OF THE REAL ELECTROCHEMICAL SURFACE AREA OF THE PELIKAN WORKING ELECTRODE.

Several studies (***80***) have shown the close relation between the active electrochemical surface area of the working electrode and the concentration range of the detected analyte (glucose). In general, high surface area electrodes are desired as the high surface area provides more active sites for electrochemical reactions, and hence higher kinetic rate capability (US patent 5728181). Therefore, the determination of the active electrochemical surface area of the electrode is very important. There are several methods, in order to calculate the real electrochemical surface area of the electrode. The principal method applied in this study was chronocoulometry, since it is a simple electroanalytical technique with many applications. (***81***)

In detail, the preliminary studies on the determination of the real electrochemical surface area of a glassy carbon electrode was carried out, followed by the real electrochemical surface studies of Acheson and Dupont screen printed electrodes, which are physically and structurally identical to the Pelikan screen printed electrodes. However, their working electrode paste was made of plain carbon paste only without enzyme (glucose oxidase), mediator (TMPD) and several buffers. The results obtained using the glassy carbon electrode were consistent, resulting in really low percentage of coefficient of variation. It was further found that there is a close relation among the concentration of the mediator, the diffusion coefficient and the

real electrochemical surface area of the electrode. By increasing the concentration of the mediator in the solution, the diffusion coefficient increased whilst the real electrochemical surface area of the electrode decreased.

The experimental performance on the actual Pelikan screen printed electrodes was investigated. After managing successfully to solve several contretemps related to the standard addition method used for calculating the unknown concentration of the mediator incorporated in the working electrode paste, we managed to optimise the method and successfully use it to determine the real electrochemical surface area of the Pelikan electrodes.

The final optimisation of the method to determine the real electrochemical surface area of screen printed electrodes was adopted by Pelikan as a potential evaluation method of their screen printed electrodes.

4.1.2 HAEMATOCRIT EFFECT-ADSORPTION OF THE BLOOD SAMPLE INTO THE WORKING ELECTRODE PASTE ACCORDING TO THE HAEMATOCRIT LEVELS

The haemetocrit differences observed in the blood samples can significantly affect glucose measurements. The most pronounced haematocrit effects occur at low and at high haematocrit levels, which generally increase and decrease glucose measurements, respectively (***82***). High heamatocrit levels up to 62.9% are presented to newborns with polycythemia and low heamatocrit levels are observed commonly to people with renal failure, hemodialysis and cardiopulmonary bypass (***83***). Several

studies have shown the possible mechanisms of the heamatocrit effects on glucose meter measurements. These mechanisms include the mechanically impede diffusion of plasma into the reagent layer, because of the increased number of erythrocytes in the whole blood sample or because of the decreased volume of plasma available to diffuse (***84***). The blood consists of plasma and particles such as the red blood cells. The viscosity of blood depends on the viscosity of plasma in combination with the heamatocrit. High heamatocrit level implies higher viscosity and consequently decrease of the fluid permeability into the reagent layer (***85***).

The reagent layer in the Pelikan Electrodes is the working electrode, consisting of the WE paste. By using a contact angle goniometer the interaction of blood samples with different haematocrit levels was observed with the WE paste. Whole blood samples with heamatocrit levels between 25 to 40 percent were tested. The results obtained showed that there is a significant difference of adsorption between the blood samples with different heamatocrit level, but the overall adsorption in accordance with time was not very significant. This adsorption can be also attributed to the hydrophobicity of the WE paste. In general, factors which influence the hydrophobicity of the surface are the nature of the material itself and the microscopic morphology of the surface (***86***). Blood samples with heamatocrit lower than 27% and consequently contact angle lower than 90^{o}, convert the surface of the WE paste to more hydrophilic, while blood samples with heamatocrit greater than 27% and consequently contact angle greater than 90^{o}, convert the surface of the WE paste to more hydrophobic. Finally blood samples with low heamatocrit can be absorbed easier, since the contact angle is lower and consequently the hydrophobicity of the surface of the WE paste, than blood samples with high heamatocrit level.

4.2 AN AMPEROMETRIC GLUCOSE OXIDASE BIOSENSOR USING AN ELECTROCHEMICALLY GENERATED CONDUCTING POLYMER

Conducting polymers (CPs) especially Polyanilines (PANIs) are receiving great attention as a result of their promising properties for optoelectronic applications such as sensors, solar cells, and light-emitting diodes (***87***), (***88***), (***89***). One of the major drawbacks of these polymers is their insoluble nature. Many studies have been performed (***90***), (***91***), (***92***) for modifying the polymer's surface, by mainly introducing functional groups onto the polymer surface in order to improve the surface hydrophilicity. Molecular design is the best mean at the moment for introducing functional groups onto polymer substrates and this can be achieved by functionalizing conjugated polymers.

The new conducting polymer N-(N, N'-diethyldicarbamoyl ethyl amido ethyl) aniline (NDDEAEA), which is characterized and acts as a matrix for the biosensor fabrication in this study, can act as a macroiniferter and is capable of electrochemical polymerisation to form PANI-based materials in various formats capable of utilization in other reactions, such as participation in free radical-initiated addition polymerisation after formation of the PANI material while preserving or even enhancing some of the PANI's properties (such as electrical conductivity) (***93***).

The basic research in this study rely on the characterisation of the NDDEAEA, the optimization of the conditions for the electrochemical polymerization, their application in grafting and finally the development of a glucose biosensor.

4.2.1 Electrochemical polymerisation of NDDEAEA

More precisely electrochemical polymerisation was used for polymerising NDDEAEA through its aniline functionality to generate conducting PANI chains. The electrochemical characterisation was carried out using cyclic voltammetry and the results obtained are quite similar to that obtained during the polymerisation of aniline and ring substituted anilines dissolved in acidic solutions. (***94***), (***95***), (***96***), (***97***), (***98***), (***99***) The large currents observed at the positive end of the CV are due to the superposition of two distinct processes: one is the electron transfer from the poly (NDDEAEA) film corresponding to the oxidation of the PANI film and the other is the electron transfer from the NDDEAEA monomer to the electrode corresponding to the oxidation of the NDDEAEA monomer to produce a precursor for the PANI film.

Iniferter-activation and photocrosslinking of electropolymerised films of NDDEAEA

When the poly(NDDEAEA)-modified electrode was irradiated with UV light in 0.75M HCl, a photochemical cleavage of the C-S bond occurred, as well as the formation of a pair of free radicals which have very different reactivities, such that a reactive carbon-centered radical and a low reactivity dithiocarbamate radical (sometimes known as dormant). This process is reversible. The end groups of the polymers can be cross-linked by only photoirradiation without the use of any chemical crosslinker such as di-isocyanates which can be harmful to the environment. Upon UV irradiation, the electropolymerized poly(NDDEAEA) generated dormant DTC radicals and active carbon centered radicals, thereby acted as an iniferter.

4.2.2 Photografting of various monomers onto electropolymerised film of NDDEAEA

In the presence of various monomers such as methacrylic acid (MAA) or styrene this allowed precise grafting with control of the macromolecular architectures of the grafted surfaces. Water contact angle measurements before and after UV irradiation in monomer solution provided evidence that the graft polymerisation proceeded only during photoirradiation.

In more details, electropolymerised films of poly(NDDEAEA) were UV irradiated in a monomer solution (MAA, styrene or AMPSA) under a nitrogen atmosphere for 20 minutes Photolysis of the iniferter component of poly(NDDEAEA)-modified electrode surfaces by UV irradiation yielded in a pair of radicals, an active carbon centered radical which can react with another monomer such as MAA or styrene to yield a radical polymer end and a dormant DTC species which reacts weakly or not at all with these monomers but can undergo to termination reactions through recombination with a growing polymeric chain. The grafted polymeric surfaces were characterized by Contact Angle (CA) measurements. Controlling the surface contact –angle through changes in the surface functionality, while retaining the bulk material properties, is useful for many applications, including sensors, flow control and cell Patterning.

By using the electropolymerised NDDEAEA with DCT groups as substrates for the surface-initiated photopolymerisation of MAA, AMPSA and styrene into homopolymers and layer by layer grafted polymer, the “living” nature of the iniferter was proven. The ‘living’ nature of the photopolymerisation has been also proved with

the formation of copolymer layers with consecutive use of two different monomers. According to several studies this type of polymeristaion can undergo difficulties regarding the photolysis of DTC end groups on poly (MAA) growing end, resulting in a less efficient grafting of second layer (***100***), (***101***), however, in this study has been confined a two dimensional photopolymerisation processes, due to the presence of high density of DTC groups; therefore the surface-growing polymer end explores only a two-dimensional space and grows in a uniform way for the first and second polymeric layer.

4.2.3 Immobilisation of glucose oxidase over electropolymerised NDDEAEA by iniferter activation using UV irradiation

There are several methods in order to immobilise the enzymes, such as adsorption, physical entrapment, crosslinking and covalent bonding. (***102***) and evethough PANI has been used not only for physical entrapment but also as a matrix for covalent enzyme immobilization, in this study has been successfully demonstrated the immobilisation of glucose oxidase onto a polyaniline derivative polymer matrix by iniferter activation using UV irradiation. The NDDEAEA/GOx electrode presented a linear response to glucose concentration within the range from 0.1mM to 5 mM with a correlation coefficient of 0.9775. A plateau current was observed when the glucose concentration was higher than 20mM. The sensitivity of the biosensor was 3.293μA mM^{-1}, while the limit of detection was 69.81μM. Regarding the interference substances it was observed that uric acid, ascorbic acid and sucrose in one to one ration with glucose affected the amperometric response to a small extend, up 8.5% for uric acid, 4.26% for ascorbic acid and 3.21% for sucrose. It is worthy to note though that the usual concentration of uric acid in the blood sample is 0.116 mM to

0.472mM, while for ascorbic acid is 0.022mM to 0.083mM. Consequently the specific interference compounds will not affect the sensor response.

4.3 DEVELOPMENT AND OPTIMISATION OF THE REACTION CONDITIONS TO GRAFTING HYPERBRANCHED POLYMER ONTO THE SURFACE OF THE MULTI-WALLED CARBON NANOTUBES

Multi-walled carbon nanotubes have attracted much attention due to their mechanical, electrical and thermal properties and their wide range of applications, including sensors, electronics, and energy storage devices. (***79***), (***103***), (***104***)

The major drawback though of the MWCNTs is their luck of solubility in organic and aqueous solutions and consequently their significant luck of exploitation in many commercial applications. By attaching small molecules or macromolecules, such as polymers, on the surface of nanotubes and by altering their functionality, scientists have overcome this problem and a significant progress in their study and application has been succeeded. (***105***)

The functionalisation of carbon nanotubes with polymers can be achieved by several techniques, such as living radical polymerisation, living anionic polymerisation and ring opening polymerisation. The living radical polymerisation is based on the covalent functionalisation of the nanotube sidewalls by two different methods, the "grafting to" and the "grafting from" methods. The "grafting to" method is achieved by attaching a preformed polymer chain onto the surface of pristine or prefunctionalised nanotubes, while the "grafting from" method, the method used in our study, is achieved by polymerising monomers from surface derived initiators on MWCNTs. (***103***)

More precisely 1,1′-Ferrocenedicarboxaldehyde and Tris(2-aminoethyl)amine were polymerised as A_2 and B_3 monomers respectively, using two different methodologies and they were then grafted onto MWCNTs following the “grafting from” approach.

A successful grafting was achieved by using the first method. As can be observed from the SEM images many tubes were aggregated to form bundles and the average diameter of the modified MWCNTs was approximately 100nm, which is approximately three times thicker than the unmodified MWCNTs. In comparison by using the second method, the SEM images obtained do not show any specific formation of polymer on the MWCNT surfaces. This is probably due to the effects of co-factors such as the monomer reactivity, the monomer concentration, or the solvent used. Several studies (***106***) have demonstrated that the reactivity of the monomer is a very important factor in grafting, since it is closely related to several factors such as the polar and steric nature, swellability of backbone in the presence of the monomers and concentration of monomers. Regarding the solvent used it is worthy to note that in general in grafting mechanisms, the solvent is the carrier by which monomers are transported to the vicinity of the backbone, so it plays a very important factor for a successful polymerisation.

5TH CHAPTER

CONCLUSION & FURTHER WORK

This project aimed at better understanding and improvements in performance of glucose biosensors, in particular these produced by Pelikan – sponsor of this work.

The first part of the study included the study of different aspects of the Pelikan screen printed electrodes. More precisely the determination of their real surface area and an investigation regarding the major haematocrit effect which most of the present commercial electrochemical glucose biosensors seem to present was carried out.

The determination of the active electrochemical surface area of the electrode is very important since high surface area electrodes are desired as the high surface area provides more active sites for electrochemical reactions, and hence higher kinetic rate capability (US patent 5728181) The principal method applied in this study was chronocoulometry, since it is a simple electroanalytical technique with many applications. (Bott et al., 2005) In detail, the preliminary studies on the determination of the real electrochemical surface area of a glassy carbon electrode was carried out, followed by the real electrochemical surface studies of Acheson and Dupont screen printed electrodes, which are physically and structurally identical to the Pelikan screen printed electrodes. However, their working electrode paste was made of plain carbon paste only without enzyme (glucose oxidase), mediator (TMPD) and several buffers. The results obtained, using the glassy carbon electrode were consistent, resulting in really low percentage of coefficient of variation. It was further found that there is a close relation among the concentration of the mediator, the diffusion coefficient and the real electrochemical surface area of the electrode. By increasing the concentration of the mediator in the solution, the diffusion coefficient

increased whilst the real electrochemical surface area of the electrode decreased. The experimental performance on the actual Pelikan screen printed electrodes was investigated and the method determing the real electrochemical surface area of screen printed electrodes was adopted by Pelikan as a potential evaluation method of their screen printed electrodes.

It is worthy to note that the close relation among the concentration of the mediator, the diffusion coefficient and the real electrochemical surface area of the electrode necessitates a further testing of the glucosensors with the actual blood sample, since all the measurements carried out in this research involved the use of buffers.

Another very important factor that can influence significantly the glucose measurements is the haemetocrit differences observed in the blood samples. The most pronounced haematocrit effects occur at low and at high haematocrit levels, which generally increase and decrease glucose measurements, respectively (***82***). The results obtained, retrieved from our study showed that there is a significant difference of absorption between the blood samples with different heamatocrit level, but the overall absorption in accordance with time was not very significant. This absorption can be also attributed to the hydrophobicity of the WE paste. In general, factors which influence the hydrophobicity of the surface are the nature of the material itself and the microscopic morphology of the surface (***86***). Blood samples with heamatocrit lower than 27% and consequently contact angle lower than 90^{o}, convert the surface of the WE paste to more hydrophilic, while blood samples with heamatocrit greater than 27% and consequently contact angle greater than 90^{o}, convert the surface of the WE paste to more hydrophobic. Finally blood samples with

low heamatocrit can be absorbed easier, since the contact angle is lower and consequently the hydrophobicity of the surface of the WE paste, than blood samples with high heamatocrit level.

The second part of the study included the characterisation of the NDDEAEA, the optimization of the conditions for the electrochemical polymerization, its application in grafting and finally the development of a glucose biosensor.

The new conducting polymer N-(N, N'-diethyldicarbamoyl ethyl amido ethyl) aniline (NDDEAEA), which was characterized and acted as a matrix for the biosensor fabrication in this study, can act as a macroiniferter and is capable of electrochemical polymerisation to form PANI-based materials in various formats capable of utilization in other reactions, such as participation in free radical-initiated addition polymerisation after formation of the PANI material while preserving or even enhancing some of the PANI's properties. More precisely electrochemical polymerisation was used for polymerising NDDEAEA through its aniline functionality to generate conducting PANI chains. The electrochemical characterisation was carried out using cyclic voltammetry and the results obtained were quite similar to that obtained during the polymerisation of aniline and ring substituted anilines dissolved in acidic solutions. (***94***), (***95***), (***96***), (***97***), (***98***), (***99***) When the was irradiated with UV light in 0.75M HCl, a photochemical cleavage of the C-S bond occurred, as well as the formation of a pair of free radicals which have very different reactivities, such that a reactive carbon-centered radical and a low reactivity dithiocarbamate radical (sometimes known as dormant). This process was reversible. Upon UV irradiation of the poly(NDDEAEA)-modified electrode, the electropolymerized

poly(NDDEAEA) generated dormant DTC radicals and active carbon centered radicals, thereby acted as an iniferter. Moving forward to photografting various monomers onto the electropolymerised poly(NDDEAEA, the “living” nature of the iniferter was successfully proven. The ‘living’ nature of the photopolymerisation has been also proved with the formation of copolymer layers with consecutive use of two different monomers.

The development of the glucose biosensor was achieved by successfully immobilising the glucose oxidase onto a polyaniline derivative polymer matrix by iniferter activation using UV irradiation. The NDDEAEA/GOx electrode presented a linear response to glucose concentration within the range from 0.1mM to 5 mM with a correlation coefficient of 0.9775. A plateau current was observed when the glucose concentration was higher than 20mM. The sensitivity of the biosensor was 3.293μA mM^{-1}, while the limit of detection was 69.81μM. Regarding the interference substances it was observed that uric acid, ascorbic acid and sucrose in one to one ration with glucose affected the amperometric response to a small extend, up 8.5% for uric acid, 4.26% for ascorbic acid and 3.21% for sucrose. It is worthy to note though that the usual concentration of uric acid in the blood sample is 0.116 mM to 0.472mM, while for ascorbic acid is 0.022mM to 0.083mM. Consequently the specific interference compounds will not affect the sensor response.

As has already been mentioned the specific compound can potentially be used in the construction of novel Pelikan electrodes with enhanced integration functionalities, e.g. grafting non-adhesive polymer coatings. In order to achieve this some further

studying need to be performed, in order to improve the sensitivity of the sensor and the effect of interferences.

The final study included the development and optimisation of the reaction conditions to grafting a hyperbranched polymer onto the surface of the multi-walled carbon nanotubes (MWCNT), using the A_3 and B_2 approach.

More precisely 1,1′-Ferrocenedicarboxaldehyde and Tris(2-aminoethyl)amine were polymerised as A_2 and B_3 monomers respectively, using two different methodologies following the “grafting from” approach. A successful grafting was achieved by using the first method, since on SEM images it was observed that many tubes were aggregated to form bundles and the average diameter of the modified MWCNTs was approximately 100nm, which is approximately three times thicker than the unmodified MWCNTs. By using the second method, the SEM images obtained did not show any specific formation of polymer on the MWCNT surfaces. This is probably due to the effect of co-factors such as the monomer reactivity, the monomer concentration, or due to the solvent used.

Since all the above factors as well as the time and temperatures of the reactions can affect the formation and grafting of the polymers onto the MWCNTs a further study is needed to be performed.

In conclusion, diabetes is one of the leading causes of death and disability in the world. As this field enters the fifth decade of intense research, the market size and the huge demand are creating the need for the development of new approaches,

allowing the development of a cheap, robust and more importantly reliable biosensor. Nowadays commercial blood glucose meters are produced by many companies worldwide, but mostly employ mediated amperometric biosensor technology. Some other alternative strategies include minimally invasive testing meters and continuous glucose monitoring. The incorporation of polymers can improve the selectivity and biocompatibility of the biosensors, while the use of hyperbranched polymers offer the chance for the development of new products, such as a variety of new nanomaterials, when grafted to multiwall carbon nanotubes. These and similar developments are expected to greatly improve the control and management of diabetes to the overall benefit of the World's population.

1. American Diabetes Association. Diagnosis And Classification of Diabetes Mellitus. *Diabetes Care* **2010,** *33,* 62-69.
2. Watkings, P. J. *ABC OF DIABETES,* 5th ed.; 2003.
3. Turner, A. P. F.; Newman, J. D.; Warner, P. J. Biosensor:A global view. *The Nonth World Congress on Biosensors* **2006**.
4. Newman, J. D.; Turner, A. P. F. Home blood glucose biosensors: A commercial prospective.*Biosensors and Bioelectronics*, **2006**, *20*, 2435-2453
5. Yoo, E.-H.; Lee, S.-Y. Glucose Biosensors:An overview of use in clinical practice. *Sensor* **2010,** *10* (5), 4558-4576.
6. Walkers, R.; Rodgers, J. Diabetes: A practical guide to managing your health.
7. American Diabetes Association. *A field guide to type 1 diabetes,* 2nd ed..
8. Tuch, B.; Dunlop, M.; Projetto, J. *Diabetes Research: A guide for postgraduates;*, 2000.
9. American Diabetes Association. *Gestational Diabetes-What to expect,* 5th ed..
10. Wild, S.; Roglic, G.; Green, A.; Sicree, R.; King, H. Global Prevalence of Diabetes.Estimates for the year 2000 and projections for 2030. *Diabetes Care*, **2004,** *27,* 1047-1053.
11. Shaw, J. E.; Sicree, R. A.; Zimmet, P. Z. Global estimates of prevalence of diabetes for 2010 and 2030. *Diabetes Research and Clinical Practice* **2010,** *87,* 4-14.
12. King, H.; Aubert, R. E.; Herman, W. H. Global burden of diabetes, 1995-2025. *Diabetes Care* **1998,** *21,* 1414-1431.
13. Rocchiccioli, J. T.; O'Donogue, C. R. Diabetes mellitus:Towards a federal policy. *Policy,Politics & Nursing Practice* **2004,** *5,* 237-242.
14. International Diabetes federation. **2004**.
15. Kirn, T. F. http://findarticles.com/p/articles/mi_7342/is_8_30/ai_62766911/ (accessed December 5, 2006).
16. International Diabetes Federation. *The Diabetes Atlas,* 2nd ed.;, 2003.
17. Turner, H. E.; Wass, J. A. H. *Oxford handbook of endocrinology and diabetes;*, 2002.

18. http://www.medicinenet.com/diabetes_mellitus/index.html/ (accessed November 28, 2006).
19. Park, H. D. Design of a portable urine glucose monitoring system for health care. *Comput.Biol.Med.* **2005,** *35,* 275-286.
20. Renald, H. D. Monitoring glycerimic control: the importance of self monitoring of blood glucose. *Amer. J. Med.* **2005,** *118,* 2-19.
21. International Diabetes Federation. *Diabetes Atlas,* 5th ed.;, 2004.
22. Johnson, R.; Baker, J. Accuracy of devices used for self-monitoring of blood glucose. *Ann. Clin. Biochem.* **1998,** *35,* 68-74.
23. Boland, E. Limitations of conventional methods of self-monitoring of blood glucose. *Diabetes Care* **2001,** *24,* 1858-1862.
24. http://www.diabetes.webmd.com (accessed november 28, 2006).
25. Margaret, G. Clinical application of "dentostix" in estimating blood glucose levels. *Diabetologia* **1996,** *1,* 245-247.
26. Drury, M. I.; Timoney, F. J.; Delaney, P. Dentrostix- A rapid method of estimating blood glucose levels. *J.Ir.Med.Assoc.* **1965,** *56,* 52-53.
27. Jensen, M. S. Clinical tests with the dentrostix. A new method for rapid blood sugar determination. *Ugeskr.Laeger* **1965,** *56,* 709-712.
28. Korp, W. A new rapid blood sugar determination at bedside (Dentrostix). *Wien. Med. Wochenschr.* **1965,** *115,* 435-437.
29. Stephenson, L. S.; Latham, M. C. Rapid and portable methods of lactose tolerance test administration. **1975,** *28,* 888-893.
30. Newman, J. D.; Tigwell, L. J.; Turner , A. P. F.; Warner, P. J. *Biosensors - A clearer view;*, 2004.
31. Wang, J. Electrochemical glucose biosensors. *Chem.Rev.* **2008,** *108,* 814-825.
32. Lee, S.-H.; Fang, H.-Y.; Chen, W.-C. Amperometric glucose biosensors based on screen printed carbon electrodes modified with hexacyanoferrate chitosan oligomers mixture. *Sensors and Actuators B* **2006,** *117,* 236-243.
33. Ransay, G.; Turner, A. P. F. Development of an electrochemical method for a rapid determination of microbial concentration and evidence for reaction mechanism. *Analytica Chimica Acta* **1988,** *215,* 61-69.

34. Turner, A. P. F.; Ramsay, G.; Higgins, I. J. Applications of electron transfer between biological systems and electrodes. *Biochem.Soc.Trans.* **1983,** *11,* 445-448.

35. Turner, A. P. F.; Karube, I.; Wilson, G. S. *Biosensors: Fundamental and Applications;*, 1987.

36. Turner, A. P. F.; Yendokimov, Y. M. *Biosensors: A Russian perspective. Advances in Biosensors;*, 1995; Vol. 67.

37. Rahman, M. M.; Ahammad, A. J. S.; Jin, J.-H.; Ahn, S. J.; Lee, J.-J. A comprehensive review of glucose biosensors based on nanostructure metal oxides. *Sensors* **2010,** *10,* 4855-4886.

38. Chaubey, A.; Malhotra, B. D. Mediated Biosensors. *Biosensors and Bioelectonics* **2002,** *17,* 441-456.

39. Emr, S. A.; Yacynych, A. M. Use of polymer films in amperometric biosensors. *Electroanalysis* **1995,** *10,* 913-922.

40. Davis, F.; Higson, S. P. J. Polymers in biosensors. In *Biomedical Polymers;*, 2007.

41. Gerard, M.; Chaubey, A.; Malhotra, B. D. Application of conducting polymers to biosensors. *Biosensors and Bioelectronics* **2002,** *17,* 345-359.

42. Barisci, J. N.; Conn, C.; Wallace, G. G. Conducting polymer Sensors. *Trens Polym. Sci.* **1996,** *4,* 301-311.

43. Cosnier, S. Biomolecule immobilisation on electrode surfaces by entrapment or attachment to electrochemically polymerised films. A review. *Biosensor and Bioelectronics* **2003,** *14,* 443-456.

44. Geetha, S.; Rao, C. R. K.; Vijavan, M.; Trivedi, D. C. Biosensing and drug delivery by polypyrrole. **2006,** *568,* 119-125.

45. Gaikwad, P. D.; Shirale, D. J.; Gade, V. K.; Savale, H. J.; Kharat, K. P.; Kakde, K. P.; Shirsat, M. D. Immobilization of GOD electrochemically synthesized PANI film by cross-linking via Glutaraldehyde for determination of glucose. *Int. J. Electrochem. Sci.* **2006,** *1,* 425-434.

46. Borole, D. D.; Kapadi, U. R.; Mahulikar, P. P.; Hundiwale, D. G. Glucose oxidase electrodes of a terpolymer poly(aniline-co-o-anisidine-co-o-toluidine) as

biosensors. *European Polymer Journal* **2005,** *41,* 2183-2188.

47. Wang, J. *Analytical Electrochemistry,* **2002**, 2nd ed..
48. Bockris, J.; Reddy, A. *Electrochemistry, ionics,* **1999**, 2nd ed.; Vol. vol.1.
49. Keizai, F. *Biosensors market, R&D, Applications and Commericial Implication;* New York, 2004.
50. Yonzon, C. R. Towards advanced chemical and biological nanosensors - An overview. *Talanta* **2005,** *67,* 438-448.
51. http://www.roche-diagnostics.com (accessed December 13, 2006).
52. http://www.invernessmedical.com (accessed December 13, 2006).
53. www.lifescan.com (accessed December 13, 2006).
54. Parkinson, G.; Pejcic, B. *Using biosensors to detect emerging infectious diseases;*, 2005.
55. http://www.abbott.com (accessed December 14, 2006).
56. http://www.bayerhealthcare.com (accessed December 14, 2006).
57. Turner, A. P. F.; Piletsky, S. Biosensors and biomimetic sensors for the detection of drugs, toxins and biological agents. In *Defense against bioterror;* Morrison, D., Milanovich, F., Ivnitski, D., Austin, T. R., Eds.;, 2005; pp 261-272.
58. The Diabetes Research in children network. Accuracy of the GlucoWatch G2 Biographer and the Continuous Glucose Monitoring System During Hypoglycemia. *Diabetes Care* **2004,** *27,* 722-726.
59. Dunn, T. C.; Eastman, R. C.; Tamada, J. A. Rates of Glucose Change Measured by Blood Glucose Meter and the GlucoWatch Biographer During Day, Night, and Around Mealtimes. *Diabetes care* **2004,** *27,* 2161-2165.
60. Schichiri, M. Needle type glucose sensor for wearable artificial endocrine pancreas. *Futura* **1985,** 197-209.
61. Pickup, J.; McCartney, L.; Rolinski, O.; Birch, D. In vivo glucose sensing for diabetes management: progress towards non-invasive monitoring. *BMJ* **1999,** *319,* 1289.
62. http://www.minimed.com/products/cgms/index.html (accessed December 15, 2006).
63. Garg, S. K.; Schwartz, S.; Eldeman, S. V. Improved Glucose Excursions Using

an Implantable Real-Time Continuous Glucose Sensor in Adults With Type 1 Diabetes. *Diabetes care* **2004,** *27,* 734-738.

64. http://currents.ucsc.edu/03-04/03-15/glucose.html (accessed December 15, 2006).
65. http://www.animascorp.com/products/pr_glucosesensor.shtml (accessed December 16, 2006).
66. Sacherer; Klaus-Dieter. Storage container for analytical devices. 6,497,854, December 24, 2002.
67. (http://www.Accu-Chek.co.uk (accessed January 12, 2007).
68. Bonner, L. M.; Desimone, J. P.; Micinski, R. J. Method and apparatus of handling multiple sensors in a glucose monitoring instrument system. 5,510,266, May 5, 1995.
69. www.bayercarediabetes.com (accessed January 15, 2007).
70. Fritz, ; List, ; Weiss, ; Deck, ; Argauer, ; Immekus,. Lancet system. 6,616,616, September 9, 2003.
71. http://www.abbottdiabetescare.co.uk (accessed January 15, 2007).
72. Weigl, B. H.; Bardell, R. L.; Schulte, T. H.; Cullen, D. C.; Demas, J. N. Coordinated efforts of mathematical modeling and experimental testing efficiently advance a design project through the prototype stage. *In vitro device technology* **2004**.
73. http://www.vscht.cz/kat/download/lab07_cyklovoltametrie_eng.pdf (accessed November 13, 2006).
74. Bott, A. W.; Heineman, W. R. Chronocoulometry. *Current Separations* **2004,** *4,* 121-126.
75. http://www.epsilon-web.net/Ec/manual/Techniques/ChronoI/ca_analysis.html (accessed February 19, 2007).
76. Moressi, M. B.; Zon, M. A.; Fermandez, H. Electrochemical and spectroscopic studies of TMPD/TMPD+ redox couple in non-aqueous binary solvent mixtures by using ultramicroelectrodes. Role of the solvation phenomenon. *Electrochimica Acta* **1997,** *42,* 303-314.
77. Ravichandran, K.; Baldwin, R. P. Phenylenediamine-Containing chemically

modified carbon paste electrodes as catalytic voltammetric sensors. *Anal. Chem.* **1983,** *55,* 1586-1591.

78. Ramsing, G.; Turner, A. P. F. Development of an electrochemical method for the rapid determination of microbial concentration and evidence for the reaction mechanism. *Analytica chimica acta* **1998,** *215,* 61-69.
79. Choi, J. Y.; Oh, S. J.; Lee, H. J.; Wang, D. S.; Tan, L. S.; Baek, J. B. In situ grafting of hyperbranched Poly(ether ketone)s onto multiwalled carbon nanotubes via A3+B2 approach. *Macromolecules* **2007,** *40,* 4474-4480.
80. Choo, L. C. S.; Liu, C. Development of a molecular imprinting thick film electrochemical sensor for cholesterol. *Sensors and actuators B* **2005,** *110,* 204-208.
81. Bott, A. W.; Heineman, W. R. Chronocoulometry. *Current Separations* **2004,** *4,* 121-126.
82. Dungan, K.; Chapman, J.; Braithwaite, S. S.; Buse, J. Glucose measurement: Confounding issues in setting targets for impatient management. *Diabetes Care* **2007,** *30,* 403-409.
83. Tang, Z.; Lee, Z. H.; Louie, R. F.; Kost, G. J. Effects of heamatocrit levels on glucose measurements with handheld meters for point of care. *Arch. Pathol. Lab. Med.* **2000,** *124,* 1135-1140.
84. Dacombe, C. M.; Dalton, R. G.; Goldie, D. J. Effect of packed cell volume on blood glucose estimations. *Arch. Dis. Child.* **1982,** *56,* 789.
85. Adamson, A. W. In *A taxtbook of physical chemistry;* Academic Press: New York, 1973; pp 339-437.
86. Suzuki, H.; Nashida, N.; Yanagimachi, I. Evaporative concentration of analytes and sensing using a super hydrophobic surface. **2004,** 712-715.
87. Malkaj, P.; Dalas, E.; Viteratos, E.; Sakkopoulos, S. pH electrodes constructed from polyaniline/zeolite and polypyrrble/zeolite conductive blends. *Journal of Applied Polymer Science* **2006,** *101* (3), 1853-1856.
88. Mu, S.; Chen, C.; Wang, J. The kinetic behavior for the electrochemical polymerization of aniline in aqueous solution. *Synthetic Metals* **1997,** *88* (3), 249-254.

89. Novak, P.; Muller, K.; Santhanam, K. S. V.; Haas, O. Electrochemically active polymers for rechargeable batteries. *Chemical Reviews* **1997,** *97* (1), 207-281.

90. Sevil, U. A.; Guven, O.; Suzer, S. Spectroscopic investigation of onset and enhancement of electrical conductivity in PVC/PANI composites and blends by γ-ray or UV irradiation. *Journal of Physical chemistry B* **1998,** *102* (20), 3902-3905.

91. Bhat, N. V.; Joshi, N. V. Investigation of the properties of polyacrylamide-polyaniline composite and its application as a battery electrode. *Journal of Applied Polymer Science* **1993,** *50* (8), 1423-1427.

92. Tao, Y.; Zhao, J. H.; Wu, C. X. Polyacrylamide hydrogels with trapped sulfonated polyaniline. *European Polymer Journal* **2005,** *41* (6), 1342-1349.

93. Lakshmi, D.; Whitcombe, M. J.; Davis, F.; Chianella, I.; Piletska, E. V.; Guerreiro, A.; Subrahmanyam, S.; Brito, P. S.; Fowler, S. A.; Piletsky, S. A. Chimeric polymers formed from a monomer capable of free radical, oxidative and electrochemical polymerisation. *Chem. Commun.* **2009,** 2759-2761.

94. Lakshmi, D. . Laksmi, D.; Whitcombe, M.J.; Davis, F.; Chianella, I.; Piletska, E.V.; Guerreiro, A.; Subrahmanyam, S.; Brito, P.S.; Fowler, S.; Piletsky, S.A.. Electrochemical sensor for catechol and dopamine based on a catalytic molecularly imprinted polymer-conducting polymer hybrid recognition element. *Analytical Chemistry* **2009,** *81* (9), 3576-3584.

95. Bidan, G. . Genies, E.M., Penneau, J.F., Poly(2-propylaniline): an electroactive polymer, soluble in organic medium in the reduced state. *Journal of Electroanalytical Chemistry* **1989,** *271* (1-2), 59-68.

96. Diaz, A. F. . Logan, J.A., Electroactive polyaniline films. *Journal of Electroanalytical Chemistry* **1980,** *111* (1), 111-114.

97. Schomburg, K. C. . McCarley, R.L. Surface-confined monomers on electrode surfaces. 11. Electrochemical and infrared spectroscopic characteristics of aniline-terminated alkanethiol monolayers on Au electrochemically treated in nonaqueous media. *Langmuir* **2001,** *17* (6), 1993-1998.

98. Hayes, W. A. Shannon, C. Electrochemistry of surface-confined mixed monolayers of 4-aminothiophenol and thiophenol on Au. *Langmuir* **1996,** *12* (15), 3688-3694.

99. Ulgut, B. . Zhao, Y., Grose, J.E., Ralph, D.C., Abraña, H.D Electrochemical properties of self-assembled monolayers of polyaniline: Effects of the thiol substituent and reduced dimensionality. *Langmuir* **2006,** *22* (9), 4433-4437.

100. Dika Manga, J. Polton, A., Tardi, M., Sigwalt, P.. Mechanism of the polymerization of n-butyl acrylate initiated by N,N-diethyldithiocarbamate derivatives. Part 1. Photolysis of butyl-2-(N,N-diethyldithiocarbamyl)propionate and oligomerization of butyl acrylate. *Polymer International* **1998,** *45* (1), 14-21.

101. Richard Turner, S. Blevins, R.W. Photoinitiated block copolymer formation using dithiocarbamate free radical chemistry. *Macromolecules* **1990,** *23* (6), 1856-1859.

102. Cosnier, S. Biomolecule immobilization on electrode surfaces by entrapment or attachment to electrochemically polymerized films. A review. *Biosensors and Bioelectronics* **1999,** *14* (6), 569-578.

103. Homenick, C. M.; Lawson, G.; Adronov, A. Polymer grafting of carbon nanotubes using free radical polymerisation. *Polymer reviews* **2007,** *47,* 265-290.

104. Terrones, M. Carbon nanotubes:synthesis, properties, electronic devices and other emerging applications. *Int. Mater. Rev.* **2004,** *49,* 325-377.

105. Liu, P. Modifications of carbon nanotubes with polymers. *Eur. Polym. J.* **2005,** *41,* 2693-2703.

106. Bhattacharya, A.; Mirsa, B. N. Grafting: a versatile means to modify polymers. Techniques, factors and applications. *Prog. Polym. Sci.* **2004,** *29,* 767-814.

107. Jarzqbek, G.; Borkowska, Z. On the real surface area of smooth solid electrodes. *Ekcrochimica Acta* **1997,** *42,* 2915-1918.

108. Freitas, R. A. Basic Capabilities. *Nanomedicine* **1999,** *1*.

109. Trojanowicz, M. Application of conducting polymers in chemical analysis. *Microchim. Acta* **2003,** *143,* 75-91.

110. Pauiliukaite, R.; Brett, C. M. A.; Monkman, A. P. Polyaniline fibres as electrodes.: Electrochemical characterisation in acid solutions. *Electrochimica Acta* **2004,** *50,* 159-167.

111. Laksmi, D.; Whitcombe, M. J.; Davis, F.; Chianella, I.; Piletska, E. V.; Guerreiro,

A.; Subrahmanyam, S.; Brito, P. S.; Fowler, S.; Piletsky, S. A. Chimeric polymers formed from a monomer capable of free radical,oxidative and electrochemical polymerisation. *Chem.Commun.* **2009,** 2759-2761.

112. Lijima, S. Helical microtubule of graphitic carbon. *Nature* **1991,** *354,* 56-58.
113. Qiu, J. D.; Zhou, W. M.; Guo, J.; Wang, R.; Liang, R. P. Amperometric sensor based on ferrocene-modified multiwalled carbon nanotube nanocomposites as electron mediator for the determination of glucose. *Analytical Biochemistry* **2009,** *385,* 264-269.
114. Wang, H.; Zhou, J.; Yu, H.; Peng, F.; Yang, J. High sensitivity glucose biosensor based on Pt electrodeposition onto low density aligned carbon nanotubes. *Int. J. Electrochem.Sci.* **2008,** *3,* 1258-1267.
115. Rusling, J. F.; Forster, R. J. Electrochemical catalysis with redox polymer and polyion-protein films. *Journal of Coloid and Interface Science* **2003,** *262,* 1-15.
116. Mano, N.; Fernandez, J. L.; Kim, Y.; Shin, W.; Bard, A. J.; Heller, A. Oxygen Is Electroreduced to Water on a "Wired" Enzyme Electrode at a Lesser Overpotential than on Platinum. *Am.Chem.Soc.* **2003,** *125,* 15290-15281.
117. Li, J.; Wang, Y. B.; Qiu, J.; Sun, D. C.; Xia, X. H. Biocomposites of covalently linked glucose oxidase on carbon nanotubes for glucose biosensor. *Bioanal.Chem.* **2005,** *383,* 918-922.

Book Chapter published in:

Handbook of Optical Sensing of Glucose in Biological Fluids and Tissues

Commercial Biosensors for Diabetes

Vasiliki Fragkou and Anthony P.F Turner
Cranfield Health, Cranfield University, Silsoe, MK45 4D, UK

Abstract: Diabetes is a metabolic disorder, which occurs either because of a lack of insulin or because of the presence of factors that oppose the action of insulin. There is currently no cure for diabetes and the control of elevated blood glucose levels, without causing abnormally low levels, is the major goal in treating diabetes. Reasonable control is now relatively easily achieved with a blood glucose meter / glucose biosensor. Currently, there are more than 40 commercialised devices for detecting blood glucose levels. As a result of intense competition, companies have resorted to a strategy where existing technology has been pragmatically optimised to meet commercial objectives, such as small size and fast response time. As a consequence of this continual development, commercial practice often differs from the theoretical papers appearing in the literature. This chapter will focus on commercial biosensors and the underlying science behind their functionality.

Paper awaiting to be published in Macromolecules Journal

Conjugated Polymers with Pendant Iniferter Units - Versatile Material for Grafting

Petya K. Ivanova-Mitseva [a], Vasiliki Fragkou [a], Dhana Lakshmi [a]1, Michael J. Whitcombe [a], Frank Davis [a], Antonio Guerreiro [a], Joe A. Crayston [b], Diana K. Ivanova[a] , Petar A. Mitsev[c] , Elena V. Piletska [a], and Sergey A. Piletsky [a]

[a] Cranfield Health, Vincent Building, Cranfield University, Cranfield, Bedfordshire, MK43 0AL, United Kingdom, [b] School of Chemistry ,University of St Andrews ,North Haugh ,St Andrews , Fife , KY16 9ST , Scotland , United Kingdom , [c] School of Engineering, Cranfield University, Cranfield, Bedfordshire, MK43 0AL, United Kingdom.

ABSTRACT: A novel compound *N*-(*N*,*N'*-diethyldithiocarbamoyl ethyl amido ethyl)-aniline (NDDEAEA) was synthesized and fully characterized. Conjugated poly-NDDEAEA, consisting of *N*-substituted polyaniline (PANI) backbones with dithiocarbamate ester pendant groups (iniferter) was synthesized by chemical and electrochemical polymerization. UV-initiated living polymerization was used to graft styrene, methacrylic acid (MAA), lauryl methacrylate (LMAA) and acrylamido-2-methylpropane sulphonic acid (AMPSA) from the conjugated macroiniferter, deposited on various surfaces (glass, polypropylene, and polystyrene, gold electrodes). Polymeric surfaces were characterized by static contact angle measurements, XPS, SEM and AFM. This versatile new material can be used for creating materials with integrated functionalities (e.g. conductivity and molecular recognition, catalysis, controlled transport properties etc.) for application in sensors, microfluidic devices, and surface patterning.

Patent:

Title: DETERMINATION OF THE REAL ELECTROCHEMICAL SURFACE AREAS OF SCREEN PRINTED ELECTRODES

Inventors: Norbert Bartetzko , Vasiliki Fragkou, Anthony Turner , and Gregor Steiner

Priority application: Provisional - US Ser. No. 61/141,159 filed 12/29/2008

Serial No. 12/640,446 Filed: 12/27/2009

Cranfield UNIVERSITY

Mechanistic studies on ultrafast electrochemical nanobiosensors for glucose

V. Fragkou[1], A.P.F Turner[1], Y. Ge[1], D. Freeman[2], N. Bartetzko[3]
[1] Cranfield Health, Cranfield University, Silsoe, MK45 4D, UK
[2] Pelikan Technologies, Inc.1072 East Meadow Circle, Palo Alto, CA 94303, USA
[3] Pelikan Technologies GmbH & Co. KG, Lise Meitner Strabe 7, D-48161, Munster, Germany

Background

Currently, there are more than 40 commercialised devices for detecting blood glucose levels. Because of the high competition, the companies have resorted to a strategy, where the existing technology is "re-packaged" just to meet the commercial objectives, such as the small size and the fast response and consequently moving far away from the theoretical understanding of the functionality of these devices, generating a serious gap in the knowledge required for further optimisation.

Aim

The aim of this project will be to facilitate a further optimisation of the current Pelican Glucosens device, by studying and understanding the behaviour of the current generation of commercial glucose microbiosensors. Pelikan Sun™ is the first fully-integrated home blood glucose monitor with smart lancing. By the systematic exploration of leading-edge glucose microbiosensors and by using the Glucosens as the model system, we will attempt to develop a detailed understanding of each element of the biosensor prior to the device reassembling and developing the bridge between the modelling and the practical observations, which is currently lacking.

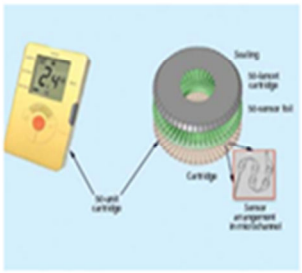


Fig 1 Pelikan Sun™

Fig 2 Screen Printer

Methodology

The systematic exploration of the different layers in the sensor when they are exposed to blood and the consequent underlying kinetics will be investigated by using such different instruments and equipments:

- Optical Microscopy
- Confocal Microscopy
- Scanning Electon Microscopy
- Video Microscopy

- Solid state NMR
- Profilometer
- Goniometer
- Screen Printer

Fig 3 Confocal Microscope

Ultimate Purpose

The basis for the development of the next generation blood glucose nanobiosensors.

References

J.D. Newman & A.P.F Turner (2005), "Home Blood Glucose Biosensors: A Commercial Perspective", Biosensors & Bioelectronics, **20** (12), 2435-2453

www.cranfield.ac.uk

SOP-Real electrochemical Surface Area Determination:

Determination of the Real Electrochemical Surface Area of the WE

PROTOCOL

Principle:

Several studies (Chou *et al*, 2005) have shown the close relation between the active electrochemical surface area of the working electrode and the concentration range of the detected analyte (glucose). In general, high surface area electrodes are desired as the high surface area provides more active sites for electrochemical reactions, and hence higher kinetic rate capability (US patent no. 5728181). Therefore, the determination of the active electrochemical surface area of the electrode is very important. The methods applied include the use of the standard addition method for the calculation of the concentration of the mediator in each electrode, cyclic voltammetry for the calculation of the diffusion coefficient and chronocoulometry for the calculation of the real electrochemical surface area of the electrodes.

Abbreviations:

PBS: Phosphate Buffer

TMPD: N,N,N',N'-tetramethyl-p-phenylenediamine, CAS:100.22.1

Diluter - solvent: 2-(1-Methoxy)propyl Acetate 97%, CAS: 108-65-6

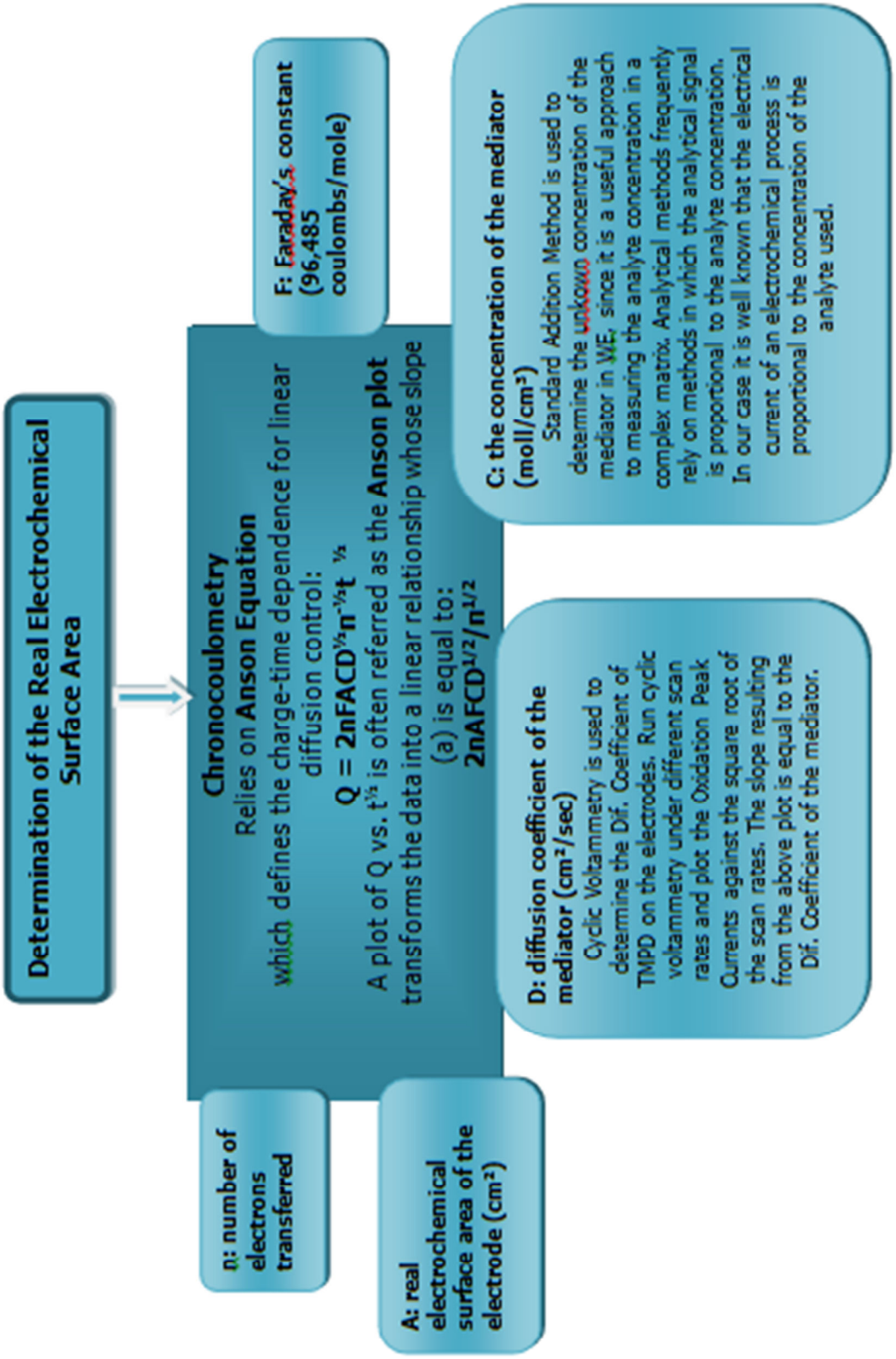
CV: cyclic voltammograms

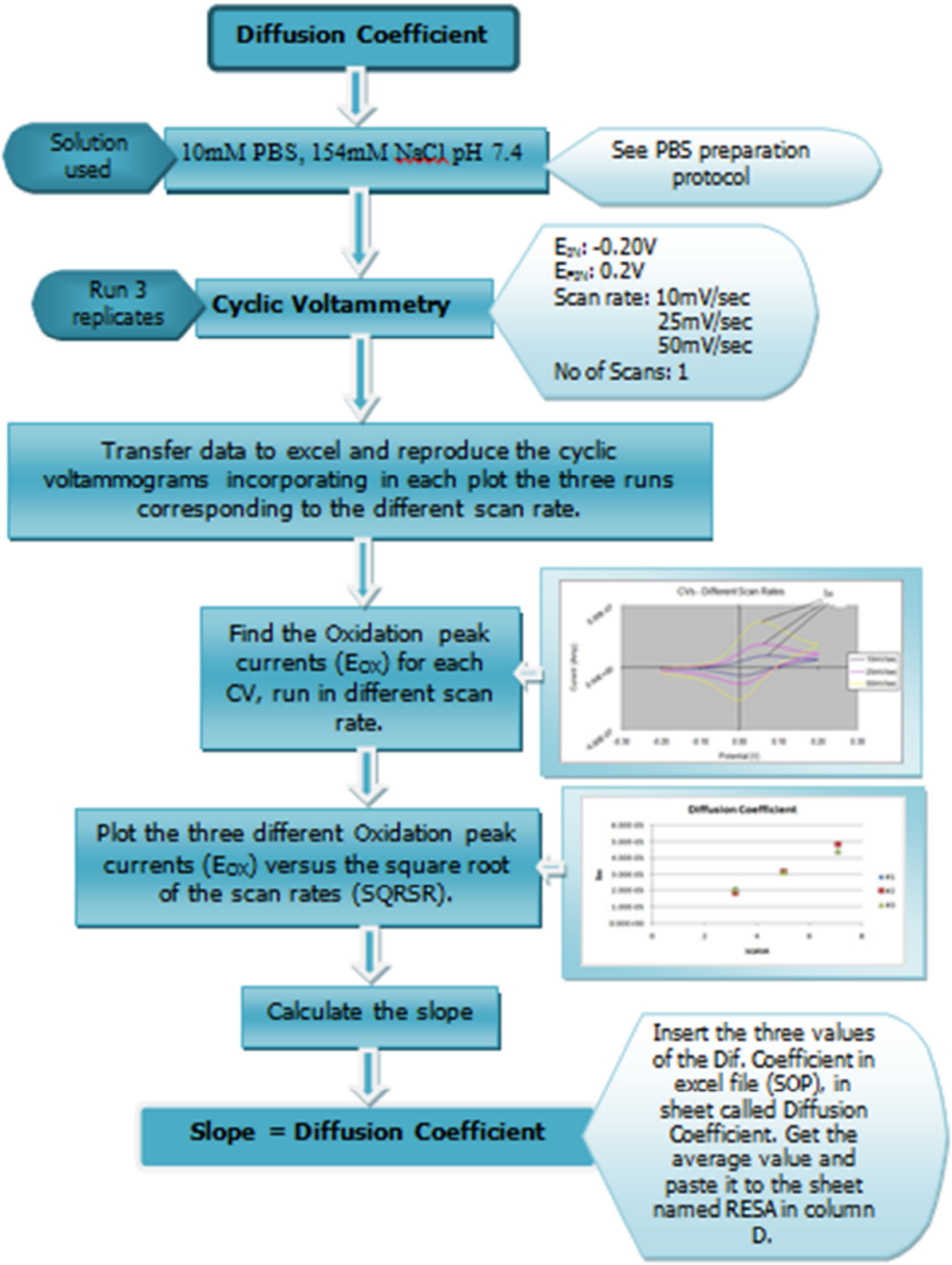
Eox: Oxidation peak current

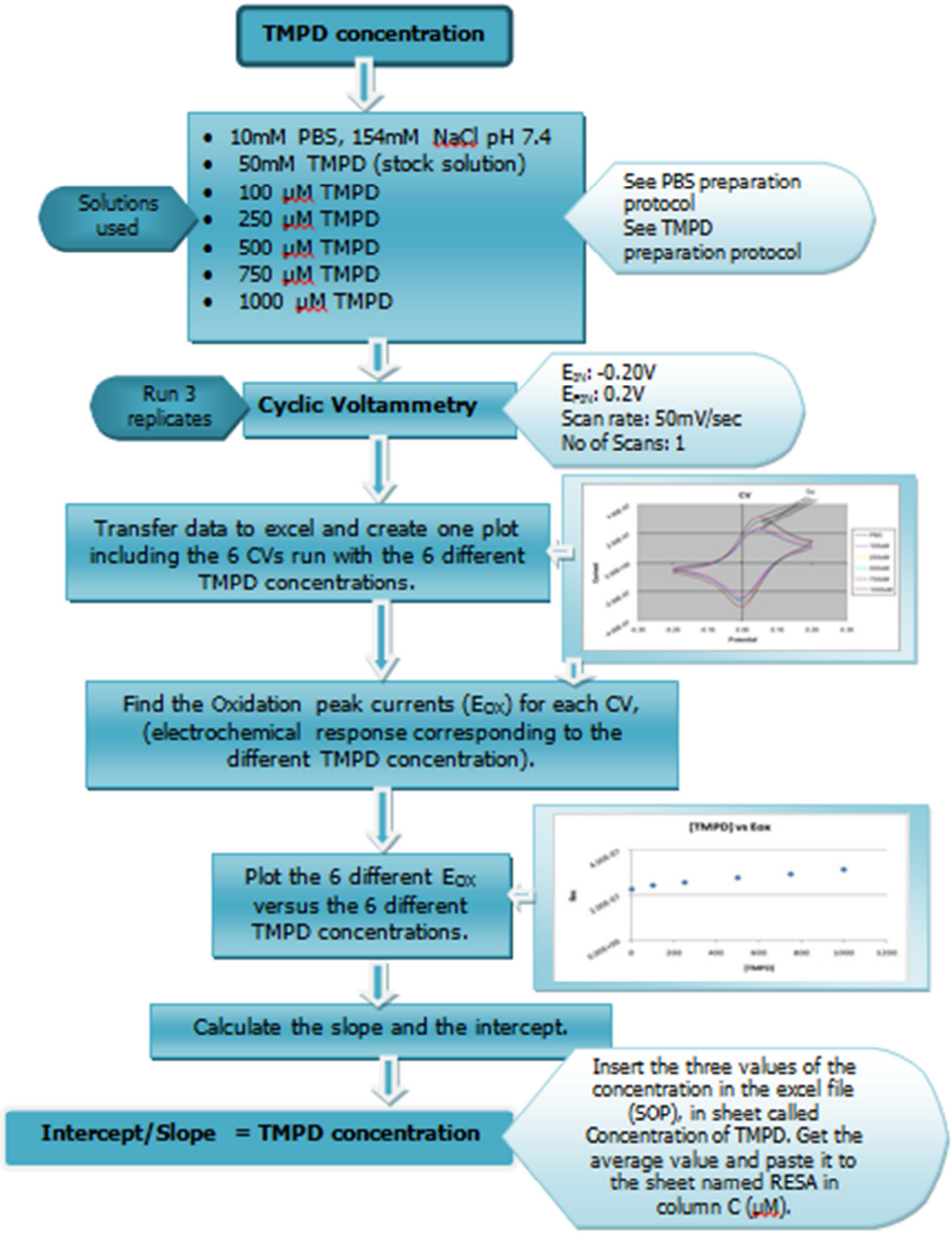
RESA: real electrochemical surface area

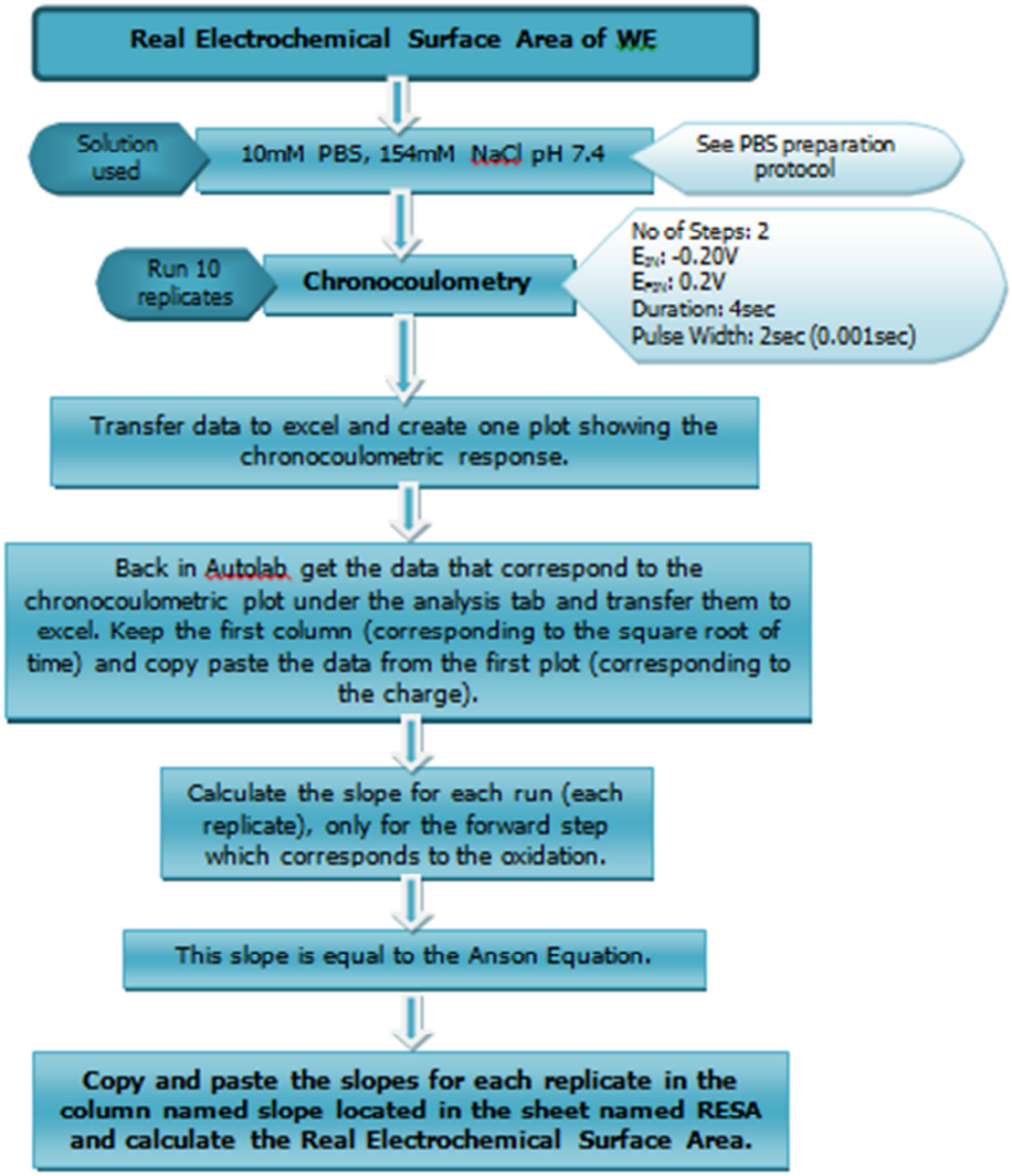
Materials:

- Sensors
- PBS: 10mM PBS,154mM NaCl, pH 7.4
- Diluter - solvent
- 50mM TMPD (stock solution)
- 100µM TMPD (in PBS)
- 250µM TMPD (in PBS)
- 500µM TMPD (in PBS)
- 750µM TMPD (in PBS)
- 1000µM TMPD (in PBS)


Determination of the Real Electrochemical Surface Area
Chronocoulometry
Relies on Anson Equation
which defines the charge-time dependence for linear diffusion control:
$Q = 2nFACD^{½}n^{-½}t^{½}$
A plot of Q vs. $t^{½}$ is often referred as the Anson plot transforms the data into a linear relationship whose slope (a) is equal to:
$2nAFCD^{1/2}/n^{1/2}$
F: Faraday's constant (96,485 coulombs/mole)
C: the concentration of the mediator (moll/cm³)
Standard Addition Method is used to determine the unkown concentration of the mediator in WE, since it is a useful approach to measuring the analyte concentration in a complex matrix. Analytical methods frequently rely on methods in which the analytical signal is proportional to the analyte concentration. In our case it is well known that the electrical current of an electrochemical process is proportional to the concentration of the analyte used.
D: diffusion coefficient of the mediator (cm²/sec)
Cyclic Voltammetry is used to determine the Dif. Coefficient of TMPD on the electrodes. Run cyclic voltammetry under different scan rates and plot the Oxidation Peak Currents against the square root of the scan rates. The slope resulting from the above plot is equal to the Dif. Coefficient of the mediator.
n: number of electrons transferred
A: real electrochemical surface area of the electrode (cm²)


Diffusion Coefficient
Solution used
10mM PBS, 154mM NaCl pH 7.4
See PBS preparation protocol
Run 3 replicates
Cyclic Voltammetry
E1V: -0.20V
E2V: 0.2V
Scan rate: 10mV/sec
25mV/sec
50mV/sec
No of Scans: 1
Transfer data to excel and reproduce the cyclic voltammograms incorporating in each plot the three runs corresponding to the different scan rate.
Find the Oxidation peak currents (EOX) for each CV, run in different scan rate.
Plot the three different Oxidation peak currents (EOX) versus the square root of the scan rates (SQRSR).
Diffusion Coefficient
Calculate the slope
Slope = Diffusion Coefficient
Insert the three values of the Dif. Coefficient in excel file (SOP), in sheet called Diffusion Coefficient. Get the average value and paste it to the sheet named RESA in column D.


TMPD concentration
10mM PBS, 154mM NaCl pH 7.4
50mM TMPD (stock solution)
100 μM TMPD
250 μM TMPD
500 μM TMPD
750 μM TMPD
1000 μM TMPD
Solutions used
See PBS preparation protocol
See TMPD preparation protocol
Run 3 replicates
Cyclic Voltammetry
Scan rate: 50mV/sec
No of Scans: 1
Transfer data to excel and create one plot including the 6 CVs run with the 6 different TMPD concentrations.
Find the Oxidation peak currents (EOX) for each CV, (electrochemical response corresponding to the different TMPD concentration).
Plot the 6 different EOX versus the 6 different TMPD concentrations.
[TMPD] vs Eox
Calculate the slope and the intercept.
Intercept/Slope = TMPD concentration
Insert the three values of the concentration in the excel file (SOP), in sheet called Concentration of TMPD. Get the average value and paste it to the sheet named RESA in column C (μM).


Real Electrochemical Surface Area of WE
Solution used
10mM PBS, 154mM NaCl pH 7.4
See PBS preparation protocol
Run 10 replicates
Chronocoulometry
No of Steps: 2
E1: -0.20V
E2: 0.2V
Duration: 4sec
Pulse Width: 2sec (0.001sec)
Transfer data to excel and create one plot showing the chronocoulometric response.
Back in Autolab get the data that correspond to the chronocoulometric plot under the analysis tab and transfer them to excel. Keep the first column (corresponding to the square root of time) and copy paste the data from the first plot (corresponding to the charge).
Calculate the slope for each run (each replicate), only for the forward step which corresponds to the oxidation.
This slope is equal to the Anson Equation.
Copy and paste the slopes for each replicate in the column named slope located in the sheet named RESA and calculate the Real Electrochemical Surface Area.